BIOREMEDIATION STUDY
FOR THE SANTA SUSANA FIELD LABORATORY

FINAL REPORT

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Santa Susana Field Laboratory Soil Treatability Studies
Task IV. Bioremediation Study

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Executive Summary:

This bioremediation study is one of five soil treatability studies commissioned by the US Department of Energy (DOE) as part of a larger remediation effort for Area IV of the Santa Susana Field Laboratory (SSFL); Area IV is also referred to as the site in this document. Collectively, the purpose of these studies is to support the evaluation of methods for reducing the volume of contaminated soils that may need to be removed from Area IV by excavation, hauling, and disposal methods. Bioremediation is the process of biological degradation of contaminants in the environment, typically mediated by bacteria and/or fungi. Contaminants of interest (COIs) at the site which could potentially be amenable to biodegradation include petroleum hydrocarbons, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated dioxins/furans. One of the other treatability studies examined the possibility of natural attenuation of the COIs, and the literature review in that study identified many species of bacteria and fungi which are known to biodegrade the COIs. The purpose of this study was to conduct site-specific research to determine the potential for biodegradation to reduce COI concentrations in site soils, either under natural conditions present at the site, or under enhanced conditions through the use of biostimulation (adding fertilizers, surfactants, etc.) and/or bioaugmentation (adding microorganisms known to degrade the contaminants). This research included both field testing to determine if COI-degrading bacteria and fungi are present in the soil at the site and a laboratory microcosm experiment to examine biodegradation rates under controlled conditions. The microcosm experiment used soil collected from the site to examine biodegradation under natural attenuation conditions expected to prevail at the site and investigate the use of fertilizers and other amendments for increasing biodegradation rates.

The field testing employed a combination of traditional culturing techniques and an array of molecular methods to identify microorganisms in the soil at the site which might be capable of biodegrading the COIs. Thirty soil samples were collected from the site with ranges of concentrations of each of the COIs and assayed for biodegrading microorganisms.

For direct culturing of microorganisms in the field, a model chemical was selected to represent each COI and added to agar plates containing no added carbon source other than the model compounds. The model chemicals were No. 2 diesel fuel for petroleum hydrocarbons, naphthalene for PAHs, PCB #1 (monochloro) for PCBs, and dibenzofuran for dioxin. Once cultured, DNA sequencing (16S or ITS) was used to positively identify these microorganisms. Forty five microorganisms were isolated from the culturing experiments, which included 14 unique bacterial species and 7 unique fungal species. Of these bacterial species, 10 have been reported as COI biodegraders or belong to genera that contain reported COI biodegraders such as *Pseudomonas, Arthrobacter, Streptomyces, Micromonospora,* and *Variovorax.* Of these fungal species, five have been shown to biodegrade the COIs, and several strains of the white-rot fungus *Phanerochaete chrysosporium* were cultured from site soils. These fungi in particular are reported to be capable of PCB and dioxin biodegradation under aerobic conditions.

The molecular methods used to identify microorganisms at the site included quantitative polymerase chain reaction (qPCR), terminal restriction fragment length polymorphism (TRFLP), and metagenomic sequencing of DNA extracted from site soils. qPCR quantifies specific microorganisms and genes associated with biodegradation of soil contaminants. TRFLP is a DNA analysis method that provides a genetic snapshot of the microbial community by quantifying fragments of DNA associated with different bacterial and fungal species. Metagenomics uses mass sequencing of large amounts of DNA to provide information on the taxonomic diversity of microorganisms present in an environmental sample. For these analyses, DNA was extracted directly from 30 soil samples and amplified using polymerase chain reaction (PCR). The 30 soil samples were chosen to provide a wide range of concentrations of each COI with the hope that populations of specific bacteria could be correlated with specific COIs (the qPCR analysis was only conducted on two soil samples). The qPCR analysis revealed that site soils contain significant populations of microbes that can biodegrade petroleum hydrocarbons aerobically. Genes associated with anaerobic petroleum hydrocarbon, anaerobic PAH, and aerobic PAH degraders were not detected. The qPCR technique was also used to test for the presence of the bacterium *Dehalococcoides* because this is one of the few bacterial species known to anaerobically dechlorinate PCBs and chlorinated dioxins. Only a small amount of *Dehalococcoides* was detected in only one sample, indicating that anaerobic bacterial biodegradation of these COIs is not likely to be occurring at the site. However, fungal species such as *Phanerochaete chrysosporium* were identified through direct culturing in many samples, and biodegradation of PCBs and chlorinated dioxins by such fungi under aerobic conditions has been reported. DNA analysis using TRFLP, did not reveal any correlations between particular microbial species and the concentrations of COIs in the soil. However, the TRFLP analysis did indicate great biodiversity in the microbial populations of the soil samples, which suggests a healthy microbial community at the site. Similarly, the metagenomic sequencing showed no correlation between taxonomic profiles and COI concentrations.

For the laboratory microcosm experiments, soil was collected from three locations at the site and incubated in sealed glass jars (with adequate oxygen) for 8 months (12 months for petroleum hydrocarbon measurement). One set of microcosms was run without amendments to estimate current natural attenuation rates at the site, and other sets were used to examine rates with biostimulation by adding nitrogen and phosphorus, rice hulls, and/or biosurfactant (soya lecithin). Another set was augmented with the white-rot fungus *Phanerochaete chrysosporium* to test the efficacy of bioaugmentation using this species. Gamma-irradiated microcosms served as sterilized controls. Five replicate microcosms of each treatment were used to provide a statistical basis for the analysis. Soil samples were collected and analyzed for extractable fuel hydrocarbons (EFH), PAHs, dioxins, and PCBs after 0, 4, and 8 months of incubation. EFH was additionally sampled after 12 months of incubation.

Soil COIs in the microcosms initially consisted of heavily chlorinated dioxins and PCBs, longer-chain petroleum hydrocarbons (21-40 equivalent carbon chain length), and PAHs with 4-6

aromatic rings. Small decreases in PAH, PCB, and dioxin soil concentrations were observed over the time period of the microcosm experiments, but these decreases were not statistically significant at the 95% confidence level. Addition of fertilizer and/or rice hulls (for bulking and improved aeration) did not improve biodegradation rates of PAHs, PCBs or chlorinated dioxins.

EFH analyses at 8 months were conducted by a different laboratory (Lancaster) than the laboratory (EMAX) that analyzed the initial and 4-month samples, and the soil EFH concentrations determined by this second laboratory were 4 to 5 times higher than that reported by the first laboratory for the initial samples. This difference is likely due to differences in integration methods between the laboratories. Therefore, 12-month samples were sent to the first laboratory (EMAX), and the resulting EFH concentrations reported fell in a similar range as the earlier EFH analyses. Essentially no overall change in EFH concentrations were observed for the three unamended soils over the 12-month incubation period. Microcosms amended with nutrients exhibited about a 50% decrease in EFH concentration, suggesting that biostimulation with fertilizer could improve biodegradation rates at the site. Slight decreases in EFH concentrations were observed for other amendments, such as bioaugmentation with white-rot fungi and bulking with rice hulls, but these changes were not significant at the 95% confidence level.

Preliminary mass-spectrometer analysis of EFH in these soils in the Cal Poly laboratory indicates that a significant amount of natural organic material (NOM) may be contributing to erroneously high values of EFH because these NOMs elute during the same time frame as petroleum hydrocarbons. Further study of the effect of NOM on EFH analyses of site soils is underway at Cal Poly.

The low biodegradation rates observed in the microcosm experiment are likely due the extensive weathering of the COIs at the site due to 20-50 years of on-going natural attenuation processes. Weathering of contaminants through the processes of volatilization and/or biodegradation of the most easily biodegraded compounds can leave the more recalcitrant compounds (either original compounds or degradation products) in the soil over time. Because petroleum hydrocarbons in the site soils are primarily longer-chain hydrocarbons in the C21 to C40 equivalent carbon range, it is likely that lighter hydrocarbons had been preferentially degraded, leaving the more recalcitrant longer-chain hydrocarbons in the soil. Similarly, the large PAHs (4-6 rings) may be somewhat recalcitrant and may take a long time to biodegrade. The limited PCB biodegradation was also not surprising because the PCBs detected at the site are heavily chlorinated, and bacterial biodegradation of these highly chlorinated compounds is reported to occur only under anaerobic conditions which were not observed in the field or in microcosms. Similarly, the most prevalent chemical form of the chlorinated dioxin present in the soils was octachlorodibenzodioxin (OCDD), which is the most chlorinated form of dioxin. Like PCBs, this compound requires anaerobic conditions for reductive dechlorination. However, fungi could potentially biodegrade PCBs and chlorinated dioxins under aerobic conditions. Indeed, total chlorinated dioxin concentrations

decreased in the microcosms amended with the fungi *Phanerochaete chrysosporium*, but this additional decrease was not statistically significant at the 95% confidence level.

Another effect of weathering is the sequestration of contaminants into the pore structure of the soil which can lower the bioavailability of contaminants to the microorganisms responsible for biodegradation. In some cases, researchers have found that adding surfactants to soil can release contaminants from the soil structure and improve bioavailability. In this study, addition of the natural surfactant soy lecithin to microcosm soils improved biodegradation slightly, but this effect was not statistically significant at the 95% confidence level.

In summary, while the field testing indicated the presence of bacterial and fungal species known to biodegrade the COIs, the laboratory microcosm experiments indicated that the biodegradation rates are low even with biostimulation and bioaugmentation. Nonetheless, the total time frame of the microcosm experiments was only one year or less, and more biodegradation could be expected over a longer time period. Future pilot tests could be conducted in the field to test biostimulation methods over a longer time frame and under conditions more closely matching those in the field. Given the observed improvement in biodegradation of petroleum hydrocarbons observed with nutrient addition and the slight improvement of biodegradation of PCBs and dioxins with bioaugmentation with *Phanerochaete* and addition of soy lecithin, these active bioremediation methods should be considered and may be worthy of field testing. Bioremediation may be most suitable for locations at the site where long-term biodegradation is acceptable, such as in areas with low COI concentrations or areas with limited public exposure, and where the length of time required for reaching cleanup levels would not be an issue.

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1.0. Introduction

This bioremediation study is one of five soil treatability studies commissioned by the US Department of Energy (DOE) as part of a larger remediation effort for Area IV of the Santa Susana Field Laboratory (SSFL); Area IV is also referred to as the site in this document. Collectively, the purpose of these studies is to support the evaluation of methods for reducing the volume of contaminated soils that may need to be removed from Area IV by excavation, hauling, and disposal methods. Bioremediation is defined as the process of biological degradation of contaminants in the environment, typically mediated by bacteria and/or fungi. Contaminants of interest (COIs) at the site which could potentially be amenable to bioremediation include petroleum hydrocarbons (measured as total petroleum hydrocarbons; TPH), polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated dioxins/furans. One of the other treatability studies examined the possibility of natural attenuation of the COIs, and the literature review in that study identified many species of bacteria and fungi which are known to biodegrade the COIs. However, it was not known from that study if such microorganisms are present in site soils or if conditions at the site are favorable for biodegradation of the COIs. The purpose of this study was to conduct site-specific research to determine the potential for biodegradation to reduce COI concentrations in site soils, either under natural conditions at the site, or under enhanced conditions through the use of biostimulation (adding fertilizers, surfactants, etc.) and/or bioaugmentation (adding microorganisms known to degrade the contaminants).

The potential for bioremediation at the site was investigated through a combination of field tests to determine if COI-degrading bacteria and fungi are present in the soil at the site and a laboratory microcosm experiment to examine biodegradation rates under controlled conditions. The field testing employed a combination of traditional culturing techniques and an array of DNA analyses to identify microorganisms in the soil at the site which might be capable of biodegrading the COIs. The microcosm experiments used soil collected from the site to examine biodegradation under natural attenuation conditions expected to prevail at the site and also evaluate the use of fertilizers and other amendments for increasing biodegradation rates.

For the field testing, 30 soil samples were collected from the site with ranges of concentrations of each of the COIs and assayed for biodegrading microorganisms. Direct culturing of microorganisms in the field was conducted by growing soil isolates on agar plates with model chemicals selected to represent each COI with no other added carbon source except the model compounds. The model compounds used were No. 2 diesel fuel for petroleum hydrocarbons, naphthalene for PAHs, PCB #1 (monochlorobiphenyl) for PCBs, and dibenzofuran for dioxin. Once cultured, DNA sequencing (16S or ITS) was used to positively identify these microorganisms. The isolated and identified bacteria and fungi were compared to known

biodegraders of the COIs to determine if the site soils contained microorganisms capable of biodegrading the COIs.

In addition to direct culturing, an array of DNA-based molecular methods were used to examine microorganisms growing in soil at the site. These methods do not require culturing and therefore are capable of identifying microorganisms (or their enzymatic functions) without the need to grow the microorganisms on plates. This is important because researchers have reported that most soil bacteria are not able to be grown in the laboratory on agar plates (Amann et al. 1995). For these assays, DNA was extracted directly from the 30 soil samples and amplified using polymerase chain reaction (PCR). Three different DNA analysis methods were employed: Quantitative PCR (qPCR), terminal restriction fragment length polymorphism (TRFLP), and a metagenomic mass sequencing of ribosomal RNA. The descriptions of these methods and rationales for the use of each of these methods are described below.

The qPCR analysis (Udvardi et al. 2008) of the DNA samples was used to quantify populations of specific microorganisms known to biodegraded the COIs and also quantify specific gene targets associated with such biodegradation. Specifically, genes associated with aerobic and anaerobic petroleum-hydrocarbon and PAH biodegradation were chosen as target genes to assay with this technique. The qPCR analysis was conducted by Microbial Insights, Inc. Their QuantArray® Petro analysis included 18 targets for biodegradation of petroleum hydrocarbons and PAHs, and biphenyl dioxygenase which is involved in PCB biodegradation. The qPCR technique was also used to test for the presence of the bacterium *Dehalococcoides* because this is one of the few bacterial species known to anaerobically dechlorinate PCBs and chlorinated dioxins.

DNA analysis using terminal restriction fragment length polymorphism (TRFLP) was used to examine the soil microbial community as a whole so that effects of COIs on the microbial population dynamics could be observed. The TRFLP method provides the relative abundance of microbial species (Kaplan and Kitts, 2004), and it was hoped that this information could be used to identify microorganisms with higher populations associated with high concentrations of each COI. Such correlations could potentially be used to infer biodegradation of the COI by the microorganisms with increased populations. Comparison of observed TRFLP patterns to libraries of TRFLP patterns for known microorganisms were also used to infer the presence of certain types of microorganisms. The TRFLP analyses was conducted at Cal Poly in the Center for Applications in Biotechnology Lab.

The metagenomics assay used mass sequencing of ribosomal RNA (rRNA) to fully characterize the microbial community of the site soil samples. This method provides the relative abundance of bacterial populations down to the genus (and sometimes species) level. An attempt was made to correlate these populations with COI concentrations as described above for the TRFLP analysis. The metagenomic rRNA gene sequencing analysis was conducted by Sandia National Laboratories in Livermore, CA.

For the laboratory microcosm experiments, soil was collected from three locations at the site and incubated in sealed glass jars. Stoichiometric calculations indicate that the air space in the microcosm jars could provide adequate oxygen for COI biodegradation. One set of microcosms was run without amendments to estimate current natural attenuation rates at the site, and other sets were used to examine rates with biostimulation by adding nitrogen and phosphorus, rice hulls, and/or biosurfactant (soya lecithin). Another set was augmented with the white-rot fungus *Phanerochaete chrysosporium* to test the efficacy of bioaugmentation using this species. Gamma-irradiated microcosms served as sterilized controls. Five replicate microcosms of each treatment were used to provide a statistical basis for the analysis. Soil samples were collected and analyzed for extractable fuel hydrocarbons (EFH), PAHs, dioxins, and PCBs after 0, 4, 8 and 12 months of incubation.

Site conditions were also assessed to determine the suitability for bioremediation and to determine appropriate conditions for the laboratory microcosms. In particular, soil vapor analyses were conducted at the site to determine the availability of oxygen in the sub-surface soil environment. This is important because some types of biodegradation, such as that of petroleum hydrocarbons, require aerobic conditions, while others, such as bacterial dechlorination of PCBs and chlorinated dioxins, require anaerobic conditions. Soil temperatures at the site were also monitored to determine the temperature to use for laboratory incubation of the microcosms.

Together, the results of the field and laboratory studies were used to estimate the potential for biodegradation of the COIs under current site conditions and also evaluate the potential for biostimulation and bioaugmentation to increase biodegradation rates of the COIs at the site. A companion study on phytoremediation was also conducted at Cal Poly to investigate potential contributions of plants to bioremediation.

2.0. Background/Literature Review

2.1. Site Background

A variety of activities at the site led to soil contamination with various chemicals ("Boeing: Santa Susana" 2014). The SSFL was established in 1947 by North American Aviation for testing liquid-propulsion rocket engines. SSFL was divided into four different areas, and the Department of Energy (DOE) performed research in a section of Area IV named the Energy Technology Engineering Center (ETEC). During the ETEC's operation, the soil was contaminated with petroleum hydrocarbons, polyaromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), chlorinated dioxins/furans, and heavy metals which together are referred to as the contaminants of interest (COIs). After the closure of ETEC, the DOE was responsible for the cleanup of soil in Area IV. The DOE commissioned this study of phytoremediation as one of five soil treatability studies that were designed to support the evaluation of methods for reducing the volume of contaminated soils that may need to be removed from Area IV by traditional excavation, hauling, and disposal methods (Sandia National Laboratories 2012). The five studies included: bioremediation, natural attenuation, phytoremediation, soil partitioning, and mercury characterization. For the bioremediation study, Sandia recommended the following treatment study tasks: Determine what biota/microbiota are currently present in Area IV soils; the rate of biologic degradation, if any, for the various contaminants in the affected soils; what nutrients/additives can be used to stimulate/increase native biota/microbiota degradation rates (i.e. biostimulation); and what non-native biota/microbiota could be used to degrade existing contaminants without interfering with native biota.

2.2. Site Soil Contamination

Previous site assessment indicates that COI concentrations in Area IV soils span a wide range (Table 2.1). Concentrations of longer equivalent carbon chain hydrocarbons are significantly higher than concentrations of their shorter-chain counterparts (Table 2.1). This suggests that lighter hydrocarbons have likely already degraded (a preferential substrate to microorganisms). The longer equivalent carbon chain hydrocarbons that are left behind are highly weathered and likely bound to soil particles, reducing their bioavailability (Smith et al. 2007).

Most of the PAH soil contamination found at the site is comprised of compounds with 4-6 aromatic rings (Appendix F). This observation agrees with our literature review which indicates that PAH degradability becomes more difficult as the number of aromatic rings increases. Thus, as observed for hydrocarbons, the PAHs with fewer rings may have already biodegraded, leaving more recalcitrant PAHs with more aromatic rings.

Table 2.1: Historic Area IV contaminant soil concentration ranges.

Contaminant Type	Contaminant	Low Concentration	High Concentration
TPH (ppm)	Heavy lube oil	170	82,000
	Diesel (31-40 carbons)	5.9	5,100
	Diesel (20-30 carbons)	31	1,300
	Gasoline	3	6.6
	Kerosene (15-20 carbons)	0.44	350
SVOC/PAH (ppm)	SVOC/PAH	0.0063	351
Dioxins (TCDD TEQ, ppt)	Dioxins	$2.7x10^{-6}$	6.5×10^{-4}
PCBs (ppb)	Aroclor 1242	392	
	Aroclor 1248	34	24,000
	Aroclor 1254	19	9.1
	Aroclor 1260	4.2	49

Soil PCB contamination at the site consists primarily of heavily chlorinated Aroclor mixtures (Aroclors 1254, 1260, and 5460). These Aroclors are 54%, 60%, and 59% chlorine by weight, respectively. The predominance of these heavily chlorinated PCB mixtures' at the site compared to their lighter chlorinated counterparts and the site's aerobic soil gas data support the well-cited literature hypothesis that more heavily chlorinated PCBs require anaerobic conditions to degrade to lighter-chlorinated compounds. Initial site characterization indicates that lesser-chlorinated PCBs either were not used at the site or have been aerobically degraded (Appendix G), and more heavily chlorinated PCBs remain in soil because of the aerobic conditions.

The majority of chlorinated dioxin contamination at the site is composed of the octachlorodibenzodioxin (OCDD) congener, which is the most heavily chlorinated dioxin. The next most common dioxin is 1,2,3,4,6,7,8 heptachlorodibenzo-p-dioxin (HpCDF), but it is present at levels less than 10% that of OCDD concentrations soils used in these experiments (Appendix E). The toxic equivalent factor (TEF) of individual compounds and the resulting dioxin toxicity equivalence (TEQ) must be understood when assessing the site contamination. The two predominant dioxin compounds at the site have fairly low TEF values: OCDD's TEF is 0.0003, and HpCDD's is 0.01 (EPA 2013a).

In 2005 the Topanga Wildfire burned almost all of the brush on the site and the surrounding Simi Hills. The fire burned 24,000 acres, including 2,000 of the 2850 acres of SSFL (roughly 80% of

the site) (ETEC 2005; Chew 2006). Some buildings sustained substantial fire damage, about 10 out of the 200 on the site. During the fire, roughly 150 pounds of Freon® were lost from air-conditioning units (ETEC, 2005). The effects of the fire on the contaminants are largely unknown. However, fires are known to produce dioxins, particularly when the fuel source has high chlorine concentrations (Thomas and Sprio 1994). Due to the large release of Freon (a chlorinated compound) during the fire, it is possible that more dioxins were produced (ETEC 2005).

More detailed descriptions of the COIs can be found in the Cal Poly Natural Attenuation Report submitted to DOE in 2014 (Nelson et al. 2014). That report also includes published biodegradation rates of each of the COIs in both field and laboratory studies.

2.3. Bioremediation Technologies

2.3.1. Monitored Natural Attenuation

Monitored natural attenuation (MNA) is used to monitor or test the progress of existing natural attenuation processes that can degrade contaminants in soil and groundwater. It can be useful if the degradation rates are fast enough to protect both human health and the environment (EPA 2013). In some cases, natural attenuation is as effective as more complex bioremediation technologies. For example, Couto et al. (2010) conducted a study that showed natural attenuation was as efficient as bioaugmentation, surfactant addition, and nutrient supplementation at remediating oil-contaminated soils at one particular site. However, site conditions and planned future use are likely to have a significant effect on the effectiveness of natural attenuation *vs*. more active bioremediation strategies.

2.3.2. Biostimulation

The term biostimulation encompasses several remedial technologies used to enhance biodegradation in the field by supplementing soils with growth substrates and/or co-substrates. Popular biostimulation agents include bulking materials (for increased aeration), nutrient supplementation, halogenated priming compounds (halopriming), and surfactants (Rastegarzadeh, Nelson, and Ririe 2006; Richardson et al. 2012; Harkness et al. 1993; Couto, Monteiro, and Vasconcelos 2010; Krumins et al. 2009; Lawniczak, Marecik, and Chrzanowski 2013; Mukherjee and Das 2010; Mulligan, Yong, and Gibbs 2001; Neu 1996; P. K. S. M. Rahman and Gakpe 2008; Rust and Wildes 2008; Fava et al. 2004; Kobayashi et al. 2012; Llado et al. 2013; Providenti et al. 1995; Tiehm et al. 1997a; Soeder et al. 1996; Rodriguez-Escales et al. 2013; Aronstein and Paterek 1995; Yong-lei et al. 2011; Viisimaa et al. 2013; Inakollu, Hung, and Shreve 2004; Whang et al. 2009; K. S. M. Rahman et al. 2002; Gorna et al. 2011).

Nutrient supplementation can be an effective biostimulation method when biodegradation of contaminants is nutrient-limited. A review of the literature indicated that nutrient

supplementation effectively enhances biodegradation of petroleum hydrocarbons, PCBs (lightly chlorinated congeners) and PAHs (Harkness et al. 1993; Couto, Monteiro, and Vasconcelos 2010; Richardson et al. 2012). However, even if a carbon source is readily available, microbial growth may be inhibited by limited microelement availability (Lawniczak, Marecik, and Chrzanowski 2013), and thus biostimulation with micronutrient addition could be useful in some situations.

Halopriming, a method by which halogenated compounds are added to soils already contaminated with halogenated compounds, has been shown to improve bioremediation of PCBs. Through addition of pentachloronitrobenzene to PCB-contaminated soils, concentrations of lesser-chlorinated PCB congeners (2-4 chlorines per biphenyl) increased by 20 ± 1.9% after 415 days of incubation (Krumins et al. 2009).

2.3.3. Bioaugmentation

Bioaugmentation involves the addition of microorganisms known to biodegrade contaminants to soil or groundwater at a contaminated site. In many cases the indigenous microbial populations are well suited for biodegradation, but in some cases bioaugmentation with bacteria and/or fungi can increase biodegradation rates at a site (Bento et al. 2005).

For the current site study, two types of bioaugmentation are of interest. One is bioaugmentation with bacteria capable of dechlorinating highly chlorinated compounds such as PCBs and chlorinated dioxins. For example, the bacterium Dehalococcoides has been shown to be useful for reductive dechlorination (Bunge et al., 2003; Fennell et al. 2004; Krumins et al. 2009). However, reductive dechlorination requires anaerobic conditions which are not likely to be present in site soils.

Bioaugmentation was explored in this study using a species of white-rot fungus because these fungi are a promising class of fungi that have been shown to degrade many of the recalcitrant contaminants found at SSFL. For example, Takada et al. (1996) showed that the fungi *Phanerochaete sordida* substantially degraded tetra- to octa-chlorodibenzo-p-dioxins (PCDDs) and tetra- to octa-chlorodibenzofurans (PCDFs). *Pleurotus ostreatus*, another white-rot fungi, degraded PCBs in a study using wood chips as the primary fungus growth substrate (Zeddel et al.1993). After five weeks, a PCB-congener mixture of primarily tri- and tetra-chlorinated biphenyls at 2500 ppm was degraded more than 95%. The fungi *Phanerochaete chrysosporium* has also been shown to biodegrade many of the COIs, although some strains work better than others (Pointing, 2001).

Bioaugmentation for degrading petroleum hydrocarbons has been fairly successful (for example Malina and Zawierucha 2007; Lee, Kang, and Cho 2011), although some studies have found biostimulation to be more effective than bioaugmentation (for example Abdulsalam et al. 2011). Bioaugmentation has also produced mixed results for PAH biodegradation. In one study,

bioaugmentation increased biodegradation of pyrene and phenanthrene by 68% and 86%, respectively, in aged soils compared to biostimulation (S. Hwang and Cutright 2002). Another study indicated that native soil microbiota hampered augmented microorganisms' growth in petroleum hydrocarbon and high-molecular weight PAH-contaminated soil (Llado et al. 2013).

Consideration should be taken prior to amending soils with foreign microorganisms, though. One study showed that antagonistic effects were observed for native soil microbiota when PAH-contaminated soils were augmented with non-native white-rot fungi (Llado et al. 2013).

2.3.4. Surfactant Addition

Surfactants are amphiphilic molecules that can increase bioavailability of hydrophobic compounds that are embedded in the soil matrix. They work by increasing a compound's solubility in the aqueous phase (Lawniczak, Marecik, and Chrzanowski 2013; Inakollu, Hung, and Shreve 2004; Whang et al. 2009). They may also change cell membrane properties and increase microbial adherence, increasing the likelihood of direct substrate uptake when two immiscible phases are present (Neu 1996; Franzetti et al. 2009). Surfactants tend to deposit at the oil/water interface (Lawniczak, Marecik, and Chrzanowski 2013).

Both synthetic (petrochemical) and natural (oleochemical) surfactant sources are available. Primary petrochemical surfactant feedstocks are crude oil derivatives such as ethylene and benzene. Typical oleochemical surfactant feedstocks are seed oils (palm, soybean, and coconut oils), but plant carbohydrates and animal fats may be used as well. There are four types of surfactants available: anionic, nonionic, cationic, and amphoteric. The largest group, anionic surfactants, has superior wetting and emulsifying properties and tends to be constituted of higher-foaming materials (Rust and Wildes 2008).

Biosurfactants are surfactants formed by microorganisms, and they are known to rival their synthetic counterparts' efficiency while being more biodegradable and less toxic to contaminant-degrading microorganisms (Lawniczak, Marecik, and Chrzanowski 2013). They may either be added to soils externally (most common) or produced onsite. For onsite production, soils must either contain or be augmented with microorganisms capable of biosurfactant production (Lawniczak, Marecik, and Chrzanowski 2013). Rhamnolipids are a type of biosurfactant that has been widely used for stimulating biodegradation (Rahman et al. 2002). For example, rhamnolipid surfactants accelerated degradation of petroleum hydrocarbons in a study by Inakollu, Hung, and Shreve (2004). However, these researchers indicated that the use of biosurfactants enhanced biodegradation of all hydrocarbons except phenanthrene and naphthalene, perhaps because surfactant solubilization is influenced by contaminant molecular size and structure. However, researchers have reported successful use of other surfactants to enhance mobilization and biodegradation of PAHs in soils (Tiehm et al. 1997b). Some nonionic surfactants were able to enhance degradation of naphthalene and phenanthrene as observed by Aronstein et al (1991).

The largest volume of soy-based surfactants is constituted by soy lecithin, an anionic surfactant (Rust and Wildes 2008). It has been shown to improve biodegradation of both PCBs and PAH (Fava et al. 2004; Soeder et al. 1996).

Biological and chemical surfactants are very promising remedial amendments for PCB-contaminated soils: addition of biological and chemical surfactants resulted in 47-50% PCB removal in one study (Viisimaa et al. 2013), biosurfactant amendment reduced concentrations of hexa- to nona-chlorinated congeners by 10-20% in another study (with no significant change in overall PCB concentrations), and the biosurfactant soya lecithin, specifically, resulted in 40% degradation of all PCBs in one year (Federici et al. 2012).

Before surfactants are applied in the field, several factors must be considered: cost, effectiveness at low concentrations (generally less than 3%), low toxicity, low adsorption to soil, low soil dispersion, and low surface tension. All of these factors should be considered prior to surfactant selection (Mulligan, Yong, and Gibbs 2001). An important consideration when applying biosurfactants for bioremediation of contaminants is the bio-compatibility between the contaminants, microorganisms, and biosurfactants. Native microflora may also impact *in-situ* biosurfactant treatment. Rhamnolipids can sometimes be biodegraded preferentially over contaminants (Chrzanowski et al. 2012). For example, Lin et al. (2011) showed initial enhanced biodegradation diesel oil through addition of biosurfactants, but the biodegradation rate in latter stages of the study was similar to that in the absence of biosurfactants.

2.3.5. Combined Treatments

Biostimulation and bioaugmentation can be combined with each other and other technologies to successfully accelerate contaminant degradation even more than from one treatment alone. For example, one study assessed both bioaugmentation and biostimulation to accelerate dechlorination of chlorinated dioxins (Bedard, Ritalahti, and Loffler 2007). Another study indicated that the combination of biostimulation and bioaugmentation in a silty-loam soil with 60,600 mg/kg of a complex mixture of petroleum hydrocarbon contaminants (comprised of 40% aliphatic hydrocarbons and 21% PAHs) was more effective than biostimulation alone (Mancera-López et al. 2008). In this study, bioaugmentation with *Rhizopus* sp., *Penicillium funiculosum* and *Aspergillus sydowii* resulted in 36%, 30% and 17% more PAH degradation compared to biostimulation alone, respectively. Another 120-day study indicated that a combined treatment using biostimulation, biosurfactant, and bioaugmentation resulted in the highest hydrocarbon degradation rate of the five treatments assessed (biostimulation, biosurfactant addition, bioaugmentation, natural attenuation, and the combined treatment) (Bento et al. 2005). Similar results were obtained in another study where bioaugmentation combined with nutrient and surfactant amendments resulted in 50% TPH degradation, while natural attenuation resulted in just 30% TPH degradation (Couto, Monteiro, and Vasconcelos 2010). A study by the author found the best biodegradation rates for petroleum compounds in drill cutting muds through the

use of bulking agents, fertilizer and inoculation with soil microbes (Rastegarzadeh, Nelson and Ririe 2006).

3.0. Field Assays of the Soil Microbial Community

3.1. Field Assay Methods

3.1.1. Soil Sample Location Selection

Soil samples were collected from 30 locations within Area IV for use in direct culturing and the DNA-based assays. This number of sample locations was selected after consideration of statistical needs and budget. Three sets of ten soil samples were collected from Area IV locations, each selected to provide a range of COI concentrations from low to moderately high. This was done so that the TRFLP analysis could be performed on sets of samples that spanned a range of COI concentrations. The premise was that an increase in concentration of a particular COI could be related to the increase in a specific peak indicating a specific microbe or group of microbes. One set of ten samples was collected with a range of PCB concentrations while trying to keep other COIs kept at a minimum. Another set of ten samples was collected with a range of chlorinated dioxin concentrations. The third set of ten samples was selected to provide a range of TPH and PAH concentrations (these two contaminants are co-located throughout the site so they have been combined into a single set of ten samples). The locations and COI concentrations for these soil samples are shown in the results section below.

The qPCR analysis was performed only on two soil samples due to cost. The two samples used were a composite sample from the microcosm experiments and a sample from one of the 30 locations described above (specifically, sample D03). Soil sample D03 was selected because it had high concentrations of chlorinated contaminants.

3.1.2. DNA Extraction Protocol

This DNA extraction protocol was used for several segments of the experiments. Using the Power Soil DNA Extraction Kit® (MoBio, Carlsbad, CA), 1 gram of soil sample was added to 2-mL PowerBead® Tubes (MoBio, Carlsbad, CA). The PowerBead® Tube contains anthracite beads that help break down cell membranes and buffer that disperses the soil particles, dissolves humic acids, and protects nucleic acids from degradation. For pure culture samples, approximately 20 µL of biomass from suspension was added. For each sample, this was done 3 times in 3 tubes. The products of these 3 tubes were combined later. If at the end there was less then 10 ng/µL of DNA in the final solution, then the whole procedure was repeated with 6 tubes. For samples that were re-extracted due to not enough DNA from the first extraction, only 1/4 gram of soil was used in 6 replicates. This allows more volume of reagent per gram of soil, allowing for better extraction efficiency. Samples were vortexed on high for 5 s. Solution C1 in the extraction kit was checked to make sure there was no precipitation. If there was precipitation, the solution was heated to 60°C until dissolved before use. Solution C1 contains sodium dodecyl sulfate (SDS) and other disruption agents required for complete cell lysis. In addition to aiding in

cell lysis, SDS is an anionic detergent that breaks down fatty acids and lipids associated with the cell membrane of many organisms. 60 µL of Solution C1 was added to each sample and inverted. A Fast Prep machine (Thermo Scientific) was then used to mechanically mix the soil in the tubes. The tubes were placed in a rack and clamped down. The machine then moves the rack much like a paint can mixer to introduce mechanical shaking. This step is critical for complete homogenization and cell lysis. Cells are lysed by a combination of chemical agents and mechanical shaking introduced by the Fast Prep machine. By randomly shaking the anthracite beads in the presence of disruption agents, collision of the beads with one another and with microbial cells causes the cells to break open. For soil samples, the Fast Prep machine was used to mix the samples at 5 m/s for 45 s. For pure culture samples, the Fast Prep was used to mix samples at 4.5 m/s for 30 s. Tubes were then centrifuged at 10,000 x *g* for 30 s. Between 400 to 500 µL of supernatant was transferred to a clean 2-mL microcentrifuge tube. Supernatant was occasionally dark in appearance and still contained some soil particles, particularly for clay soils. Subsequent steps in the protocol removed both soil particles and coloration of the mixture. If less than 400 µL of supernatant was produced then the samples were centrifuged again and the remaining supernatant was transferred. Again, this happened only occasionally and only with the clay soils. After centrifuging, 250 µL of Solution C2 was added to the samples and vortexed for 5 s. These samples were then incubated in the freezer for 10-15 min. Solution C2 contains a reagent to precipitate non-DNA organic and inorganic material including humic acid, cell debris, and proteins. After 15 min the samples were centrifuged for 1 min at 10,000 x g. Avoiding the pellet mass in the bottom of the tube, up to 600 µL of supernatant was transferred to a clean microcentrifuge tube. The pellet at this point contained non-DNA organic and inorganic material including humic acid, cell debris, and proteins. For the best DNA yields and quality, transferring any of the pellet with the supernatant was avoided with careful pipetting. 200 µL of solution C3 were added to each sample and vortexed for 5 s. Samples were cooled in the freezer for 10-15 min. Solution C3 is a second reagent to precipitate additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. Samples were again centrifuged for 1 min at 10,000 x g. Up to 750 µL of supernatant was transferred to a clean microcentrifuge tube. The pellet at this point contains additional non-DNA organic and inorganic material including humic acid, cell debris, and proteins. 1.2 mL of Solution C4 were added to the supernatant and vortexed for 5 s. Solution C4 is a high-concentration salt solution. Since DNA binds tightly to silica at high salt concentrations, this solution will adjust the salt concentrations to allow binding of DNA, but not non-DNA organic and inorganic material that may still be present at low levels, to the spin filters used in the next step. Approximately 675 µL of sample were loaded onto a spin filter and centrifuge at 10,000 x g for 1 min. Permeate was discarded into the Mo Bio waste container and 675 µL more supernatant was loaded on the spin filter and centrifuged at 10,000 x g for 1 min. Load the remaining supernatant onto the spin filter and centrifuge at 10,000 x g for 1 min. This was repeated until all the supernatant from all replicate tubes was filtered through the same filter. A total of three loads for each tube processed are required.

DNA is selectively bound to the silica membrane in the spin filter device in the high salt solution. Almost all contaminants pass through the filter membrane, leaving only the desired DNA behind. Once the replicates are all combined onto one filter, 500 µL of Solution C5 was added and centrifuged for 30 s at 10,000 x g. Solution C5 is an ethanol-based wash solution used to further clean the DNA that is bound to the silica filter membrane in the spin filter. This wash solution removes residues of salt, humic acid, and other contaminants while allowing the DNA to stay bound to the silica membrane. The permeate was discarded. The permeate was just non-DNA organic and inorganic waste removed from the silica spin filter membrane by the ethanol wash solution. Samples were then centrifuged again for 1 min. This second spin removes residual Solution C5 (ethanol wash solution). It is critical to remove all traces of wash solution because the ethanol in Solution C5 can interfere with many downstream steps such as PCR, restriction digests and gel electrophoresis (Complete Genomics 2013). The filter was carefully moved to a clean microcentrifuge tube. Then 100 mL of nano-pure, PCR-grade water was added to the center of the white filter membrane and incubated for 15 min. As the water passes through the silica membrane, DNA is released because it only stays bound to the silica spin filter membrane in the presence of high salt concentration. Samples were centrifuged at 10,000 x *g* for 30 s. Filters were removed and discarded. DNA was quantified using the Spectradrop spectrometer. A 4-µL (1-mm) slide cover was used. DNA was stored in a freezer at -20°C until use.

3.1.3. TRFLP Analysis of Soil Samples

DNA was extracted from each soil sample using the MoBio soil DNA extraction kit as described above and then prepped for PCR as follows.

PCR for TRFLP

Each PCR reaction well contained 10 µL of the sample's DNA extracted with the MoBio system and 40 µL of master mix. For bacterial TRF the master mix contained 10 µL 5X Buffer, 3 µL dNTPs (10 mM, 2.5mM of each, A,T,C,G), 2 µL BSA (20 µg/mL), 7 µL MgCl2 (25 mM), 1 µL labeled 8dF (10 µM) (AGAGTTTGTTCMTGGCTCAG), 0.3 µL AmpliTaq Gold (5 U/µL) and enough water to bring the total volume up to 50 µL per sample. For fungal TRF the master mix contained 10 µL 5X Buffer, 2 µL dNTPs (10mM, 2.5mM of each, A,T,C,G), 5 µL $MgCl_2$ (25mM), 1 µL labeled ITS1F (10 µM) (GTATTACCGCGGCTGCTGG), 1 µL ITS4 (10 µM), 0.3 µL AmpliTaq Gold (5 U/µL) and enough water to bring the total volume up to 50 µL per sample. Two control reactions were used for each PCR run. These controls included a closed negative (master mix, no DNA, not opened outside PCR room), and a positive (DNA known to amplify with PCR conditions). The positive controls were E. coli for bacterial samples and Pichia farinose for fungal samples. The PCR machine was set to the following cycling parameters for bacterial PCR: 94°C for 10 min, then 30 cycles (94°C for 1 min, 46.5°C for 1 min, 72°C for 2 min), then 72°C for 10 min and then 4°C soak until the samples were removed

from the machine. For fungal PCR, the following parameters were used: 94°C for 10 min, then 13 more cycles (95°C for 35 s, 55°C for 55 s, 72°C for 45 s), then 13 cycles (95°C for 35 s, 55°C for 55 s, 72°C for 2 min), then 9 cycles of (95°C for 35 s, 55°C for 55 s, 72°C for 3 min), then 72°C for 10 min and then 4°C soak until the samples were removed from the machine. After the first round of PCR, gels were run to ensure the DNA was replicated. Five µL of PCR product were added to each well on a 1.5% agarose gel. The gels ran for 20 min at 100 V and 400 mA. DNA was visualized with ethidium bromide. For samples that had successful PCR, two more rounds of PCR were done with gels to confirm DNA replication for each. For samples that had unsuccessful PCR DNA was re-extracted from culture or soil.

After the gel was visualized and it was confirmed that the PCR was successful two more PCR runs for each sample were done. These replicates were done to ensure even replication of all DNA in the samples because they are community samples.

PCR Clean Up

Using the PCR Ultra-Clean kit (MoBio, Carlsbad, CA), 5 volumes SpinBind® solution were added to each well and pipeted up and down to mix. Sample replicates were then combined into a spin filter and centrifuged for 30 s at 10,000 x *g*. Permeate was discarded into MoBio waste container. 300 mL of SpinClean® buffer was added to each spin filter and centrifuged for 30 s at 10,000 x g. Permeate was discarded into MoBio waste container. Spin filters were centrifuged for 120 s at 10,000 x g to remove any remaining fluid. Spin filters were transferred to clean 2.0-mL collection tubes. 60 mL of PCR water was added to the spin filter and incubated for 10 min. Samples were centrifuged for 60 s at 10,000 x g. Spin filters were discarded. DNA was quantified with the Spectradrop spectrometer. DNA was stored at –20°C.

DNA Digest

Based on the concentration of DNA in each sample, 30 ng of DNA was added to each well in a 96 well CEQ plate. These clear plastic plates were used in the CEQ 8000 machine in a later step. 5-10 ng of either E. coli or Pichia digest standard were used as controls. For bacterial samples 1.0 µL DpnII (10,000 U/mL) and 4 µL buffer were added per reaction. PCR grade water was added to bring the volume to 40 µL. For fungal samples, 1.0 µL of HaeIII (10,000 U/mL) and 4 µL buffer were added per reaction. PCR-grade water was added to bring the volume to 40 µL. The samples were placed in the PCR machine for 4 hr @ 37°C then cycled to either 65°C for DpnII, 65°C for HhaI, or 80°C for HaeIII for 20 min to deactivate the enzyme and finally to 4°C until they were removed from the machine. Samples were stored at -20°C until ready for ethanol precipitation.

Ethanol Precipitation

100 mL (2.5 x digest volume) of cold 95% ethanol and 2 mL 3M NaAc pH4.6 (5% digest volume) and 1 µL glycogen (20 mg/mL) were added to each digest sample in the CEQ plate. With the caps on, the plate was inverted 5 times to mix. The samples were then placed in the -20°C freezer for 30 min. Samples were then centrifuged for 15 min at 5300 RPM to pellet DNA (program 2). Prompt removal of samples from centrifuge will ensure minimal loss of sample. Samples were then inverted once to remove ethanol. 100 µL of cold 70% ethanol was then added to each sample. Samples were then centrifuged for 5 min at 5300 RPM (program 3). Ethanol was removed by inverting the PCR tray once on a paper towel. The CEQ plate was inverted on top of a paper towel, placed back in the centrifuge still inverted, and centrifuged for 1 min. @ 700 RPM to dry the pellet (program 4). DNA was stored in the -20°C freezer until ready to proceed to CEQ8000 preparation.

CEQ 8000 Sample Preparation

The CEQ 8000 (Beckman Coulter, Brea, CA) is a genetic analysis system that performs fragment analyses on digested DNA samples. It measures the relative quantity of fragments for each fragment length and produces a chromatograph. A master mix was made of 20 µL formamide and 0.25 µL 600-base-pair standard per reaction. 20 µL of the master mix was added to each tube. One drop of mineral oil was added to the top of each well to prevent sample evaporation. CEQ program was set up with each sample's name. PCR grade water was added to the tray inside the CEQ. The CEQ was run. The appropriate PPE was used during DNA extractions, PCR, PCR clean up, DNA Digest, and operating the CEQ.

TRFLP Analysis

Bacterial and fungal TRFLP data were analyzed separately. Fragment data produced by the CEQ 8000's fragment analysis were transferred to an excel spreadsheet where it was truncated to 1% using a macro program. This removes all peaks that are less than 1% of the largest peak, effectively removing the "noise" in the data. A similarity matrix was run on the data in Primer 5. Chemical data for all COIs for each site were added as "sample data". A non-metric multi-dimensional scaling analysis (MDS) was performed on the similarity matrix. This method determines non-parametric monotonic relationships between the similarities within the similarity matrix. Non-metric refers to the fact that the data does not belong to any specific distribution. Results were visualized in two dimensional scatter plots. The following factors were added to each sample location in the similarity matrix: COI series, location, soil type, presence of TPH, presence of PAHs, presence of PCBs, and presence of dioxin. COI series was defined by which sample set the sample came from (i.e. TPH/PAH, dioxin, or PCBs). The categories were T for TPH/PAH, P for PCBs, and D for dioxins. Location was selected based on the map of Area IV with the 30 sample locations overlaid. The 7 most northern locations were designated north and the 9 southern most sample locations were designated south. The remaining sample locations

were labeled central. Soil type was designated based on visual observations of the soil. Categories included sandy, sandy loam, clay, clay loam, silt, and silt loam. Presence of TPH was determined by a threshold of 350 ppm. Presence of PAHs was determined by a threshold of 2.5 ppm. Presence of PCBs was determined by a threshold of 450 ppb. Presence of dioxin was determined by a threshold of 5 ppb. The concentrations of the respective contaminants were used to generate corresponding bubbles over each sample that indicated the concentration. Factors used in MDS analysis of TRFLP data are shown in the results section below.

In addition to the MDS analysis, the Primer 5 was also used to calculate the index multivariate dispersion (MVDISP) for each factor. This index is a measure of how similar two groups of samples are. It is analogous to a p value in statistical significance testing. In order to be significant, the index of two groups must be between .05 and -.05 (Stobart et al. 2009). These indices provide a quantitative measurement the similarity of the two groups of samples.

TRFLP fragment data were also analyzed for correlations between specific peaks and COI concentrations using a correlation function in Excel. COI concentrations and fragment data for each sample were used in these correlations. Correlations between a particular fragment length and a COI were considered significant if the square of the correlation was above 0.4.

Finally, the fragment lengths of microbes isolated in the culturing experiments were searched for in the TRFLP data. For each isolate, the sequence data were imported to a Word document. The word search function was used to determine what the fragment length would be if digested with the restriction enzymes used for the TRFLP analysis. The enzyme DpnII was used on bacteria and HaeIII was used for fungi. These enzymes cut DNA at very specific sequences. Using a word search on the text file of the sequence data, a space was added in the middle of the first instance of that restriction sequence. A character count was then used to determine how long the DNA fragment would be if the restriction enzyme cut there. The fragment lengths were recorded for later comparison to TRFLP data.

For each isolate, the samples that contain its TRFLP signature as well as its relative abundance are presented in the results section below. Because of the possible error from the CEQ machine and the PCR digest, TRFLP peaks that were within one base pair of the cultured microbe's predicted fragment length were considered reported.

3.1.4. qPCR Analysis of Soil Samples

qPCR assays were conducted by Microbial Insights Inc. (Knoxville, Tennessee). As described above, two soil samples were used in the qPCR analysis. Sample 1 was from Soil Sample D03, which had just been received from the site 2 days prior to being mailed to Microbial Insights. Sample 2 was a composite sample of soil collected from three site locations for the microcosm experiment. This composite sample had been sifted with a #4 sieve (4.75 mm), and stored in a Tedlar® bag inside a 5-gallon bucket in the laboratory at room temperature for about 3 months.

For each sample approximately 100 g of soil was placed in a whirlpak® bag. These samples were shipped overnight on ice to Microbial Insights and analyzed within 7 days of receipt.

Microbial Insights performed a QuantArray Petro analysis, as well as a CENSUS analysis for *Dehalococcoides* and biphenyl dioxygenase for each of the two samples. The QuantArray Petro includes the analysis of the following targets: benzene/toluene dioxygenase (TOD), toluene/benzene monooxygenases (RMO, RDEG), phenol hydroxylase (PHE), ethylbenzene and isopropylbenzene dioxygenases (EDO, BPH4), naphthalene dioxygenases (NAH, NAG, PHN), MTBE-utilizing strain PM1, TBA monooxygenase, alkane monooxygenases, benzyl succinate synthase (BSS), benzene carboxylase (ABC), naphthalene carboxylase, (ANC), naphthylmethylsuccinate synthase (NMS), alkyl succinate synthase, benzoyl coenzyme A reductase (BCR), total bacteria (EBAC), and sulfate reducing bacteria (APS). These targets will be discussed in detail below.

QuantArray Petro Methods (from Microbial Insights):

This method uses qPCR as well, but combines it with microarrays to run numerous parallel reactions. A few nano liters of sample are added to each hole in a microarray slide. Each hole will carry out an individual qPCR reaction, and can target whatever gene is chosen. This allows for many genes to be targeted at the same time, leading to a greater amount of information about the sample. QuantArray uses discrete through-holes for each qPCR reaction which prevents compromising the reaction kinetics, which can be a problem for multiplex qPCR. qPCR reactions in this technique use primers and fluorescent markers like the CENSUS technique to select and count gene copies generated (Microbial Insights 2014a).

CENSUS Methods (from Microbial Insights):

CENSUS is a qPCR-based technique that uses fluorescent markers to count the number of gene copies generated in a PCR reaction. Each time a gene copy is made a fluorescent marker is released and measured with a detector. Primers are used to target specific genes to be duplicated. This technique is significantly more accurate than the traditional culturing methods, which can report less than 10% of a targeted microbe group leading to underestimating the population (Microbial Insights 2014b).

qPCR Targets

Per the recommendations of Microbial Insights, the analyses selected were the QuantArray® Petro and the Census: Dehalococcoides (DHC). The QuantArray® Petro included 18 targets for petroleum hydrocarbon and PAH degradation, including biphenyl dioxygenase which is involved in PCB biodegradation. Table 3.1 below summarizes the targets of the QuantArray® Petro assay and specifies the enzyme name, constituent attacked by the enzyme, constituent group, and if the enzyme is part of an aerobic or anaerobic process. The only specific bacterium target,

Methylibium petroleiphilum PM1 (PM1), is one of the few bacteria have been isolated that can use methyl tertiary butyl ether (MTBE) or tetra butyl alcohol (TBA) as a growth substrate. This bacterium uses TBA monooxygenase (abbreviated TBA in Microbial Insight reports) to break down MTBE and TBA (Hanson, Ackerman, and Scow 1999).

Table 3.1: Targets of QuantArray® Petro

Target Name	Enzyme Name	Constituent Attacked by Enzyme	Constituent Group	Aerobic/ Anaerobic
TOD	Benzene/Toluene Dioxygenase	Benzene/Toluene	TPH	Aerobic
EDO	Ethylbenzene Dioxygenase	Ethylbenzene	TPH	Aerobic
RMO	Toluene Monooxygenases	Toluene	TPH	Aerobic
RDEG	Benzene Monooxygenases	Benzene	TPH	Aerobic
TOL	Xylene/Toluene Monooxygenase	Xylene/Toluene	TPH	Aerobic
PHE	Phenol hydroxylase	Phenol	TPH	Aerobic
BPH4	Biphenyl/Isopropylbenzene Dioxygenases	Biphenyl/ Isopropylbenzene	TPH	Aerobic
NAH, NAG, PHN	Naphthalene Dioxygenases	Naphthalene	PAHs	Aerobic
PM1	MTBE-utilizing strain PM1	MTBE and TBA	TPH	Aerobic
TBA	TBA Monooxygenase	TBA	TPH	Aerobic
PHN	Phenanthrene Dioxygenase	Phenanthrene	PAHs	Aerobic
ALK	Alkane Monooxygenases	Alkanes	PAHs	Aerobic
BSS	Benzyl Succinate Synthase	Benzyl Succinate	TPH	Anaerobic
ABC	Benzene Carboxylase	Benzene	TPH	Anaerobic
ANC	Naphthalene Carboxylase	Naphthalene	PAHs	Anaerobic
ASSA	Alkyl Succinate Synthase	Alkyl Succinate	TPH	Anaerobic
BCR	Benzoyl Coenzyme A Reductase	Benzoyl Coenzyme A	TPH	Anaerobic
ASSA	Alklysuccinate Synthase	Alklysuccinate	PAHs	Anaerobic
MNSSA	Naphthylmethylsuccinate Synthase	Naphthylmethylsuccinate	PAHs	Anaerobic
APS	Sulfate Reducing Bacteria	N/A	N/A	Anaerobic
EBAC	Total Bacteria	N/A	N/A	N/A

3.1.5. Metagenomic sequencing methods

Metagenomics is a second-generation DNA sequencing method which uses mass sequencing of large amounts of DNA from an environmental sample. A metagenome is the combined genomes of all the organisms present in the sample. In this study, large numbers of 16S rRNA genes (bacteria) and 18S rRNA genes (fungi) were amplified with PCR and sequenced for each soil sample. The sequence data was then run through standard database analysis to identify organisms present and their relative abundance. These data provide information on the taxonomic diversity of microorganisms present in a soil sample with much greater detail than that provided by TRFLP analysis. This allows identification to be made to the genus-level.

The metagenomics analysis was conducted at Sandia National Laboratory (Sandia) in Livermore, CA under the direction of Todd Lane. DNA extracts from the 30 soil samples (as described above) were sent to Sandia. Methods used are described by Maphosa et al (2012). In this analysis high-throughput amplicon sequencing was performed on each sample, specifically for the V1-V3 region of the bacterial 16S rRNA gene.

3.1.6. Culturing and 16S Sequencing of Microbes from Contaminated Site Soil Media, Stock Solutions of Model Chemicals

The media used for isolating bacteria and fungi are depicted in Table 3.2. Bushnell Haas medium is a carbon-free mineral medium, which contains no carbon food source for bacteria. Carbon sources (such as COIs) can be added to it to determine if a microbe can survive on that carbon source alone. This is the main medium used for isolation in this experiment. Additionally, TSB was used to make liquid media to grow bacteria and YM media was used to grow fungi for enrichment cultures and cultivating biomass after initial isolation on spike Bushnell Haas medium.

Table 3.2: Composition and Recipes for Growth Media used in Culturing Experiments

Medium	Components	Concentration (g/L)	Final pH	Main Carbon Source	Medium Phase	Reference
Bushnell Haas	Magnesium Sulfate	0.2	7.0 +/- 0.2	none	Liquid	(Sigma Aldrich 2008)
	Calcium Chloride	0.02				
	Monopotassium Phosphate	1				
	Dipotassium Phosphate	1			Solid	
	Ammonium Nitrate	1				
	Ferric Chloride	0.05				
TSB	Enzymatic Digest of Casein	17	7.3 +/- 0.2	Dextrose	Liquid	(Acumedia 2010a)
	Enzymatic Digest of Soybean Meal	3				
	Sodium Chloride	5			Solid	
	Dipotassium Phosphate	2.5				
	Dextrose	2.5				
YM	Enzymatic Digest of Gelatin	5	6.2 +/- 0.2	Dextrose	Liquid	(Acumedia 2010b)
	Malt Extract	3				
	Dextrose	10			Solid	
	Yeast Extract	5				

To first isolate organisms that use these COIs for growth, solid or liquid carbon-free media or standard growth media were spiked with model compounds of the COIs. For liquid cultures grown in spiked media, cultures were subsequently plated out to form isolated colonies. From isolated colonies fresh, carbon-rich media was inoculated to grow enough biomass for DNA analyses. Instead of using every PAH, PCB, and dioxin for selecting for degrading organisms, model chemicals were selected based on the literature (Kyser, Hozalski, and Gulliver, 2011; Jones, Arujo, and Rodgers, 2012). The model compounds shown in Table 3.3 were used to screen for potential COI degraders by encouraging the growth of organisms that could degrade these less recalcitrant versions of the COIs. PCB #1 was selected as a model for PCBs because it is only mono-chlorinated and therefore significantly easier to degrade than higher chlorinated PCBs (Beyer and Biziuk 2009). All COI stock solutions were made with acetone, which was used to measure and distribute the COIs and was then evaporated off. These solutions were made in 50-mL centrifuge tubes and stored with secondary containment in a closed cabinet. The

concentrations of the stock solutions were 0.3 M for diesel, 0.3 M naphthalene, 0.03 M PCB 1, and 0.03 M dibenzofuran (DBZ). These stock solutions were checked to ensure that they were completely dissolved before pipetting out aliquots out for dilutions and media preparation. For each soil culture only one model chemical was used. For example, for samples that were from the dioxin set, and contained high dioxin concentration, microbes were cultured on media that contained dibenzofurans, but not naphthalene, No. 2 diesel, or PCB 1.

Table 3.3: Model Chemicals and Concentrations of Stock Solutions

COI	Model Chemical	Concentration of Stock Solution (M)
petroleum hydrocarbons	Diesel Fuel #2	0.3
PAH	Naphthalene	0.3
PCBs	PCB #1	0.03
Dioxins	Dibenzofuran (DBZ)	0.03

Inoculation Procedures: Plate Cultures

Plates were made from autoclaved TSA, YM, or Bushnell Haas medium (defined in Table 3.2). Dilutions of model chemicals were made from the stock solutions for each culturing experiment. Model chemicals did not dissolve in the liquid media, and so they would not be evenly distributed in the solid plates. Therefore the model chemicals were added to the top of the solid media. To each plate 5 mL of diluted model chemical solution was be added and spread evenly over the surface. It was assumed that the model chemicals, which were dissolved in acetone, would dissolve into the top portion of the plate, a volume of approximately 10 mL. Once 5 mL of the appropriate dilution was on each plate the lids were propped up on top of each plate in a fume hood to allow the acetone to evaporate off. Plates were checked periodically to see if liquid remained on the surface of the plate. Once the liquid was gone, the plates were allowed to sit for another 12 hr to ensure all acetone was volatilized. Negative control plates were run for each experiment to ensure all the acetone had been evaporated. These controls had clean acetone added to the top of the solid Bushnell Haas media, which was allowed to evaporate off for the same amount of time as the other plates. They were inoculated using soil from sample T01. This ensured that the acetone was completely volatilized off the plates and prevented the isolation of acetone degraders. Plates were then stored in a refrigerator until inoculation. To make an inoculum from the soil, approximately 1 g of soil was mixed into a 10-mL centrifuge tube with 9 mL of autoclaved 1% NaCl solution to create an inoculum. These tubes were vortexed for 10 s on high, then allowed to settle for 10 min. The top 0.1 mL of the inoculum was pipetted onto the plate (1/100th dilution). Sterile glass beads were then added to the plate and rolled around to spread the inoculum. After inoculation, the plates were incubated at 30°C until growth was

observed (1-5 weeks) or the experiment ended at 5 weeks. Isolated colonies that were grown on these plates were grown again in TSB or YM liquid media. DNA was then extracted from these new colonies. All plate cultures were performed in triplicate. Preparations for fungi and bacteria were identical except for the media used in the enrichment cultures. TSB was prepared for bacteria and YM was prepared for fungi enrichment cultures (Tables 3.2 and 3.3).

Inoculation Procedures: Liquid/Enrichment Cultures

Dilutions of model chemicals were made from the stock solutions for each culturing experiment (Table 3.3). Liquid cultures were prepared by adding the model chemicals in acetone to sterile 20-mL test tubes and then evaporating off the acetone. This method was adapted from experiments done by Singer, Wong, and Crowley (2002). Negative control tubes were run for each experiment to ensure all the acetone had been evaporated. These controls had clean acetone added to the tube, which was allowed to evaporate off for the same amount of time as the other tubes. Bushnell Haas media was then added to the tubes. They were inoculated using soil from sample T01. This ensured that the acetone was completely volatilized off the tubes before media was added and prevented the isolation of acetone degraders. Concentrations of added model chemicals were based on the assumptions that 5 mL of diluted model chemical solution would be added to each tube and that the final volume of each culture was to be 10 mL. Once 5 mL of the appropriate dilution was in each tube the rack was set with the caps off in a hood to allow the acetone to evaporate. Parafilm was suspended 1 inch over the tops of the tubes using an autoclaved scaffolding to minimize microbes falling into the tubes but also to allow enough air flow to evaporate the acetone in a reasonable amount of time. After the acetone was completely evaporated, 9 mL of Bushnell Haas carbon-free liquid medium or TSB or YM was added to each tube, depending on the culturing experiment. These tubes were placed in the incubator at 30°C for 1 week and then were checked for growth before inoculation. No growth was observed in any of the tubes before inoculation. To make an inoculum from the soil, approximately 1 g of soil was mixed into a 10-mL centrifuge tube with 9 mL of autoclaved 1% NaCl solution to create an inoculum. These tubes were vortexed for 10 s on high, then allowed to settle for 10 min. Then the top 1 mL of the inoculum was added to each tube of medium (1/10th dilution). These tubes were incubated at 30°C until growth was observed or the experiment ended (6 to 11 weeks). If growth was observed then the culture was plated onto TSA and YM plates. Isolated colonies that were grown on these plates were grown again in TSB or YM liquid media. All liquid cultures were performed in triplicate. DNA was extracted from each enrichment culture using the Power Soil DNA Extraction Kit® from MoBio and the procedure described below.

Culturing Positive Control Organisms

Bacterial and fungal positive control organisms that are known to degrade each model chemical were selected based on the literature review. These organisms were grown separately along with the microbes from the soil in every culturing experiment to determine if the concentration of the

model chemicals had toxic effects on the microbes and to ensure that they could grow in the selected media. Table 3.4 lists these control organisms.

Table 3.4: Positive Control Organisms for Culturing Experiments

Model Compound Chosen	Positive Control Organism	ATCC/DSMZ Strain Number	Reference
No. 2 Diesel	*Rhodococicus rhodochrous KUCC 8801*	ATCC: 21198	Sorkhoh et al. 1990
Naphthalene	*Paenibacillus naphthalenovorans*	ATCC: BAA-206	Daane et al. 2002
PCB #1	*Pseudomonas putida* KF715	ATCC: 700837	Hayase, Taira, and Furukawa 1990
Dibenzofuran	*Sphingomonas wittichii* RW1	DSM 6014	T. R. Miller et al. 2010
Fungal Control 1	*Phanerochaete chrysosporium*	ATCC 24725	Fernández-Sánchez et al. 2001; Fernández-Luqueño et al. 2011; Pérez-Armendáriz et al. 2012; Hammel, Kalyanaraman, and Kirk 1986; Hammel et al. 1992
Fungal Control 2	*Phanerochaete chrysosporium strain from Puerto Rico*	N/A	N/A

Two strains of Phanerochaete were used, one bought from ATCC and one which was shipped on solid medium from Puerto Rico by Dr. Raul Cano (Cal Poly). All other microbes were ordered from ATCC and arrived freeze dried, and were revived based on the instruction received with the cultures. 0.5 mL of TSB or YM media was added to the freeze dried cultures. After 30 min the 0.5 mL culture broth was added to 5 mL of the appropriate medium and incubated for 5 days at 30 ° C. Slants, plates and liquid cultures of these organisms were maintained throughout the experiment by re-plating onto the appropriate medium every 2 weeks. Colony morphology and visual observations of the cells under a microscope were used to confirm that the cultures were correct and pure. All model bacteria were grown on TSA or TSB, while fungi were grown on YM media (media described above in Table 3.2).

DNA Extraction and Sequencing

Once the microbes had been cultured in liquid media, their DNA was extracted using the MoBio DNA Power Soil Extraction Kit as described above (Section 3.3). Instead of using 1 gram of soil

for each sample, approximately 1 mL of the bottom of the liquid culture was used in each PowerBead® tube (still used 3 PowerBead® tubes per sample). After the DNA was extracted and quantitated with the Spectradrop machine, PCR was done on each sample. Master mixes were prepared for both fungal and bacterial PCR.

For bacterial PCR, the master mix contained 10 µL 5X Buffer, 2 µL dNTPs (10mM, 2.5mM of each, A,T,C,G), 2 µL BSA (20ug/mL), 4 µL MgCl2 (25 mM), 1 µL 8dF (10 µM) (AGAGTTTGTTCMTGGCTCAG), 1µL of 1525 R (10um), 23.7 µL of H2O and 0.3 µL AmpliTaq Gold (5U/ µL). Six µL of extracted DNA were added to each reaction. For bacterial PCR, the following cycling parameters were used: 94°C for 2 min, then 40 cycles of (94°C for 30 s, 46.5°C for 30 s, 72°C for 30 s), then 72°C for 7 min and then 4°C soak until the samples were removed.

For fungal PCR, the master mix contained 10 µL 5X Buffer, 2 µL dNTPs (10 mM, 2.5 mM of each, A,T,C,G), 2 µL BSA (20 µg/mL), 4 µL MgCl2 (25 mM), 1 µL ITS1F (10 µM) (GTATTACCGCGGCTGCTGG), 1 µL ITS4 (10uM), 23.7 µL of H_2O and 0.3 µL AmpliTaq Gold (5U/ µL). Six µL of extracted DNA were added to each reaction. Two control reactions were used for each PCR run. These controls included a closed negative (master mix, no DNA, not opened outside PCR room), and a positive (DNA known to amplify with PCR conditions). The positive controls are *E. Coli* for bacterial samples and *Pichia farinose* for fungal samples. For Fungal PCR the following cycling parameters were used: 94°C for 10 min, then 13 cycles of (95°C for 35 s, 55°C for 55 s, 72°C for 45 s), then 13 cycles of (95°C for 35 s, 55°C for 55 s, 72°C for 2 min), then 9 cycles of (95°C for 35 s, 55°C for 55 s, 72°C for 3 min), then 72°C for 10 min and then 4°C soak until the samples were removed.

For all PCR reactions a gel was run to ensure the DNA was replicated. 5 µL of PCR product were added to each well on a 1.5% agrose gel. The gel ran for 20 min at 100 V and 400 mA. DNA was visualized with ethidium bromide. For PCR successful samples, the PCR product cleanup was performed. Using the MoBio PCR Ultra-Clean kit, 5 volumes SpinBind solution were added to each well and pipeted up and down to mix. Sample replicates were then combined into a spin filter and centrifuged for 30 s at 10,000 x g. Permeate was discarded into MoBio waste container. Then 300 mL of SpinClean buffer was added to each spin filter and centrifuged for 30 s at 10,000 x g. Permeate was discarded. Spin filters were centrifuged for 120 s at 10 x kg to remove any remaining fluid. Spin filters were transferred to clean 2.0-mL collection tubes. 60 mL of PCR water was added to spin filter and incubated for 10 min. Samples were centrifuged for 60 s at 10,000 x g. Spin filters were discarded. DNA was quantified with the Spectradrop spectrometer. DNA was stored at –20°C.

Sequencing and Data Analysis

Approximately 50 ng of each DNA sample was shipped overnight to Sequatech in Mountain View, Ca. Primers were also mailed with the samples. For bacteria, the 46F, 530R, 530F and

1492R primers were used. For fungi the 8dF and 1525R primers were used. Sequence results were emailed back in 2 days. The sequences were then aligned using SeqMan software. Sequences were analyzed using the National Center for Biotechnology Information (NCBI) database known as BLAST (Basic Local Alignment Search Tool). The most likely identity of the organism as well as the query cover, indent, and E value were recorded. Query cover indicates the percent of the query sequence that overlaps with the subject sequence. The indent specifies the percent of the subject sequence that overlaps at the beginning of the sequence. It indicates how much sequence could have been lost due to where the primer is located on the gene. E value is a measure of random background noise. It describes the hits one can expect to see by chance when searching a database of a specific size. The closer the E value is to zero the more significant the match is.

3.2. Results of Field Microbial Assays

3.2.1. Soil Sample Locations and Contaminant Concentrations

The 30 soil sample locations used for this research are shown on the map in Figure 3.1. Chemical data for these sample locations are presented in Table 3.5. This table shows the concentrations of each COI at the 30 sample locations, as well as the total metals concentrations. Sample name, location code, and date sampled are also provided. Constituents marked "unknown" were not measured at that location.

Table 3.5: COI concentrations in soil from the 30 sample locations

TPH/ PAHs	Location Code	Date Sampled	TPH (mg/kg)	PAHs (µg/kg)	PCB (µg/kg)	Dioxins (ng/kg)	Total Metals (mg/kg)
T01	SL-291-SA6	9/1/11	1020	8390	473	4560	40700
T02	SL-012-SA5A	3/7/11	2410	3280	8.28	3360	38100
T03	SL-058-SA5A	4/14/11	121	6020	15.8	5150	97800
T04	SL-118-SA5A	3/14/11	1850	2820	7.34	3160	39000
T05	SL-116-SA5DN	6/28/11	119	5630	15.1	5450	108000
T06	SL-250-SA6	9/9/11	1190	2780	635	4100	41200
T07	SL-064-SA5A	4/22/11	119	5817	15.4	4860	118000
T08	SL-144-SA5DN	5/25/11	119	5820	15.3	4720	136000
T09	SL-063-SA5B	1/11/11	673	37.1	7.10	28.4	57800
T10	SL-104-SA6	8/7/11	538	3390	17.8	3140	45700

Table 3.5 continued

PCBs	Location Code	Date Sampled	TPH (mg/kg)	PAHs (µg/kg)	PCB (µg/kg)	Dioxins (ng/kg)	Total Metals (mg/kg)
P01	SL-040-NBZ	3/28/12	367	775	32000	6570	69400
P02	SL-062-NBZ	3/21/12	23.2	347	829	3010	38400
P03	5C_DG-556D	5/15/12	unknown	unknown	645	unknown	unknown
P04	SL-250-SA6	9/9/11	1190	2780	635	4100	41200
P05	SL-039-NBZ	3/28/12	232	403	576	2930	38500
P06	SL-291-SA6	9/1/11	1020	8390	473	4560	40700
P07	5C_DG-644	5/8/12	unknown	unknown	387	unknown	unknown
P08	5C_DG-558B	6/1/12	unknown	unknown	360	unknown	unknown
P09	5C_DG-558C	6/1/12	unknown	unknown	360	unknown	unknown
P10	5C_DG-634	4/20/12	unknown	unknown	360	unknown	unknown

Dioxins	Location Code	Date Sampled	TPH (mg/kg)	PAHs (µg/kg)	PCB (µg/kg)	Dioxins (ng/kg)	Total Metals (mg/kg)
D01	SL-273-SA5B	12/17/10	unknown	53.9	124	17200	71000
D02	5C_DG-596	5/3/12	unknown	unknown	unknown	20200	unknown
D03	SL-221-SA5A	3/29/11	70.0	2850	327	13100	129000
D04	SL-321-SA6	8/3/11	22.2	2680	14.5	7640	54300
D05	SL-040-NBZ	3/28/12	366	775	32000	6570	69400
D06	SL-229-SA6	10/21/11	unknown	2970	25.6	6470	39200
D07	SL-224-SA6	8/30/11	20	5440	25.2	6110	134000
D08	SL-068-NBZ	3/16/12	15.3	780	269	5680	81000
D09	SL-116-SA5DN	6/28/11	119	5630	15.1	5450	108000
D10	SL-058-SA5A	2/21/11	121	6020	15.8	5150	97800

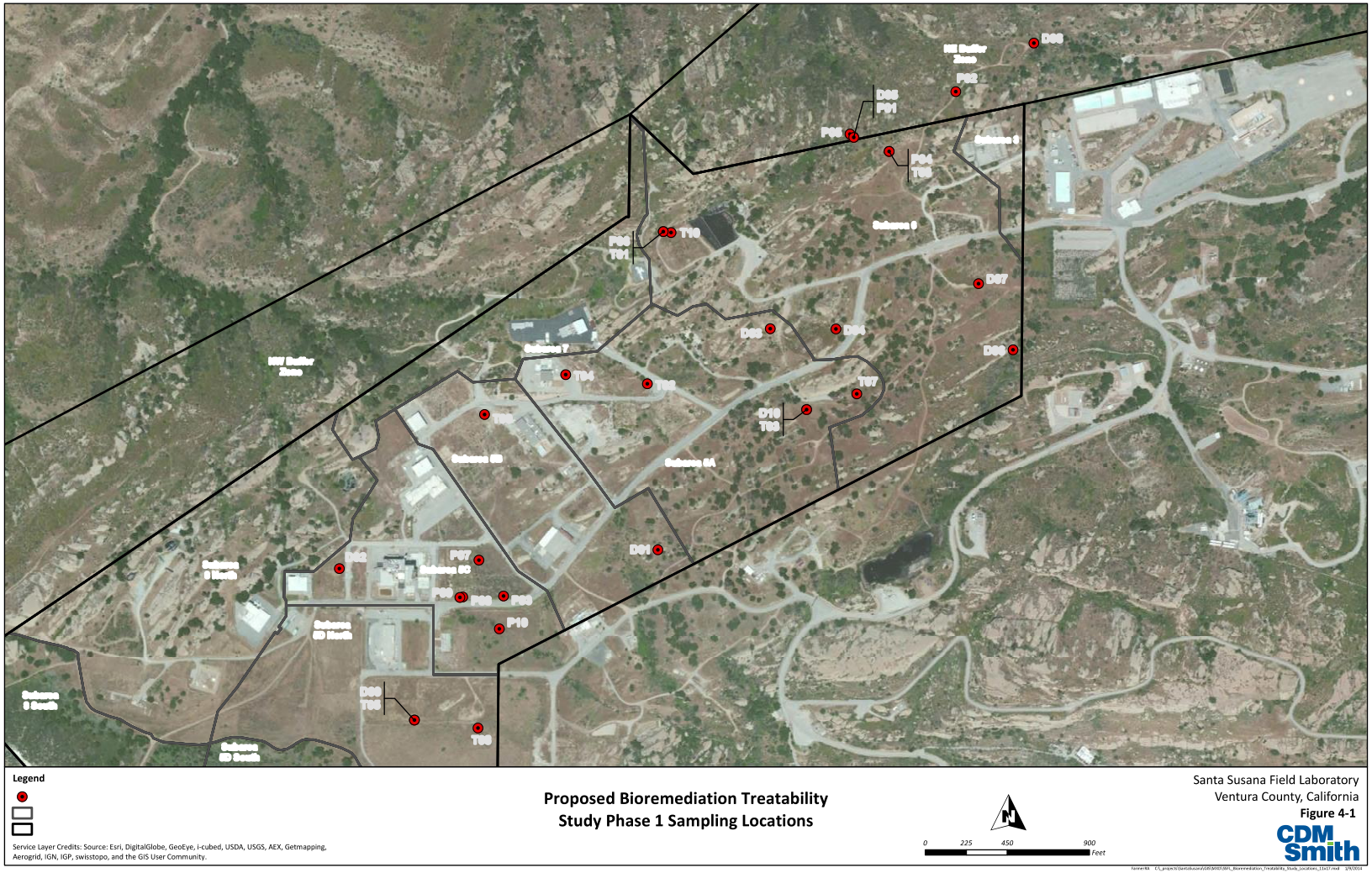
Legend
Service Layer Credits: Source: Esri, DigitalGlobe, GeoEye, i-cubed, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community.
Proposed Bioremediation Treatability
Study Phase 1 Sampling Locations
0 225 450 900
Feet
Santa Susana Field Laboratory
Ventura County, California
Figure 4-1
CDM Smith

Figure 3.1: Soil Sample Locations at Area IV (CDM Smith 2014)

3.2.2. Microbes Cultured and Identified

The conditions of the culturing experiments and the resulting cultures isolated are summarized in Table 3.6 for bacteria and Table 3.7 for fungi. From these 336 separate culturing experiments (including replicates), 45 colonies were isolated from the soil samples from SSFL. Of these 45 colonies, 9 were fungi that were isolated on solid, Bushnell Haas media that was spiked with a COI. The remaining 36 were bacterial colonies, 20 were isolated on solid, Bushnell Haas media that was spiked with a COI and 16 were isolated from TSB cultures spiked with COIs. See Figure 3.2 for examples of solid Bushnell Haas medium plates with colonies. Tables 3.6 and 3.7 (respectively) summarize how many colonies and if the colonies were bacteria or fungi for each COI.

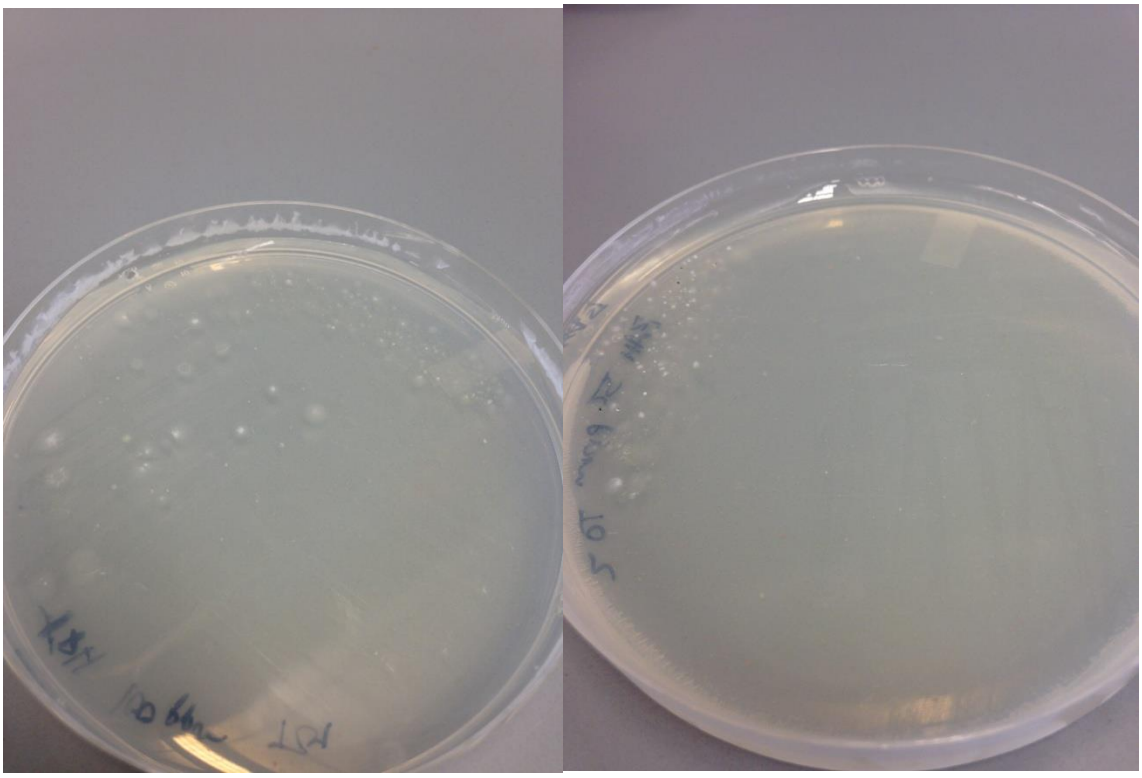

Figure 3.2: Example Plates with Colonies. The left image is a Bushnell Haas medium plate inoculated with soil from site D02 and spiked with 1 ppm of DBZ showing fungal and bacterial colonies. The right image is a Bushnell Haas medium plate inoculated with soil from site T02 and spiked with 500 ppm of naphthalene showing fungal colonies.

Table 3.6: Bacterial Culturing Conditions and Results

Notes: MCs = Model Chemicals, CFMM or CF= Carbon Free Mineral Media, TSA = Tryptone Soy Agar, TSB = Tryptone Soy Broth.
"Step" indicates the sequence of transfers from media to media within a single culturing experiment.
"x" indicates no data, because either not applicable or not attempted (in the case of the gray cells).
Bold text indicates experiment portions that yielded no growth of organisms or control organisms,

Exp #	Step #	Growth Medium	Diesel Fuel Conc (ppm)	Potential TPH Degraders Isolated	Cells/g based on Plate Counts	Naphth-alene Conc (ppm)	Potential PAH Degraders Isolated	Cells/g based on Plate Counts	PCB 1 Conc (ppb)	Potential PCB Degraders Isolated	Cells/g based on Plate Counts	DBZ Conc (ppb)	Potential Dioxin Degraders Isolated	Cells/g based on Plate Counts	Total Incu-bation Time
1	1	CFMM Plate + MCs	100	x	400	*50*	*x*	*0*	10	x	400	*10*	*x*	*0*	5 weeks
1	2	TSB	0	1	x	*0*	*0*	*x*	0	1	x	*0*	*0*	*x*	1 week
2	1	CFMM Plate + MCs	1000	x	600	500	x	1200	100	x	700	100	x	500	5 weeks
2	2	TSB	0	2	x	0	3	x	0	3	x	0	1	x	1 week
3	1	CFMM Plate + MCs	10000	x	200	5000	x	600	1000	x	500	1000	x	700	5 weeks
3	2	TSB	0	2	x	0	2	x	0	2	x	0	2	x	1 week
4	1	CFMM Plate + MCs	x	x	x	x	x	x	**10^5**	**x**	**0**	**10^5**	**x**	**0**	5 weeks
4	2	TSB	x	x	x	x	x	x	**0**	**0**	**x**	**0**	**0**	**x**	1 week
5	1	CFMM Plate + MCs	50	x	200	*10*	*x*	*0*	x	x	x	x	x	x	5 weeks
5	2	TSB	0	1	x	*0*	*0*	*x*	x	x	x	x	x	x	1 week

Table 3.6 Continued…

Exp #	Step #	Growth Medium	Diesel Fuel Conc (ppm)	Potential TPH Degraders Isolated	Cells/g based on Plate Counts	Naphth-alene Conc (ppm)	Potential PAH Degraders Isolated	Cells/g based on Plate Counts	PCB 1 Conc (ppb)	Potential PCB Degraders Isolated	Cells/g based on Plate Counts	DBZ Conc (ppb)	Potential Dioxin Degraders Isolated	Cells/g based on Plate Counts	Total Incuba-tion Time
6	1	CFMM Liquid + MCs	*100*	*x*	*x*	*50*	*x*	*x*	*10*	*x*	*x*	*10*	*x*	*x*	5 weeks
6	2	CFMM Plates + MCs	*100*	*0*	*x*	*50*	*0*	*x*	*10*	*0*	*x*	*10*	*0*	*x*	5 weeks
6	3	TSB	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
7	1	CFMM Liquid + MCs	*1000*	*x*	*x*	*500*	*x*	*x*	*100*	*x*	*x*	*100*	*x*	*x*	5 weeks
7	2	CFMM Plates + MCs	*1000*	*0*	*x*	*500*	*0*	*x*	*100*	*0*	*x*	*100*	*0*	*x*	5 weeks
7	3	TSB	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
8	1	CFMM Liquid + MCs	*10000*	*x*	*x*	*5000*	*x*	*x*	*1000*	*x*	*x*	*1000*	*x*	*x*	5 weeks
8	2	CFMM Plates + MCs	*10000*	*0*	*x*	*5000*	*0*	*x*	*1000*	*0*	*x*	*1000*	*0*	*x*	5 weeks
8	3	TSB	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
9	1	CFMM Liquid + MCs	*100*	*x*	*x*	*50*	*x*	*x*	*10*	*x*	*x*	*10*	*x*	*x*	5 weeks
9	2	TSA + MCs	*100*	*0*	*x*	*50*	*0*	*x*	*10*	*0*	*x*	*10*	*0*	*x*	5 weeks
9	3	TSB	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week

Table 3.6 Continued…

Exp #	Step #	Growth Medium	Diesel Fuel Conc (ppm)	Potential TPH Degraders Isolated	Cells/g based on Plate Counts	Naphthalene Conc (ppm)	Potential PAH Degraders Isolated	Cells/g based on Plate Counts	PCB 1 Conc (ppb)	Potential PCB Degraders Isolated	Cells/g based on Plate Counts	DBZ Conc (ppb)	Potential Dioxin Degraders Isolated	Cells/g based on Plate Counts	Total Incuba-tion Time
10	1	CF Liquid + MCs	*1000*	*x*	*x*	*500*	*x*	*x*	*100*	*x*	*x*	*100*	*x*	*x*	5 weeks
10	2	TSA + MCs	*1000*	*0*	*x*	*500*	*0*	*x*	*100*	*0*	*x*	*100*	*0*	*x*	5 weeks
10	3	TSB	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
11	1	CFMM Liquid + MCs	*10000*	*x*	*x*	*5000*	*x*	*x*	*1000*	*x*	*x*	*1000*	*x*	*x*	5 weeks
11	2	TSA + MCs	*10000*	*0*	*x*	*5000*	*0*	*x*	*1000*	*0*	*x*	*1000*	*0*	*x*	5 weeks
11	3	TSB	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
12	1	TSB Liquid + MCs	100	x	x	50	x	x	*10*	*x*	*x*	10	x	x	5 weeks
12	2	TSA	100	2	x	50	1	x	*10*	*0*	*x*	10	4	x	5 weeks
12	3	TSB	0	x	x	0	x	x	*0*	*x*	*x*	0	x	x	1 week
13	1	TSB Liquid + MCs	1000	x	x	500	x	x	100	x	x	100	x	x	5 weeks
13	2	TSA	1000	2	x	500	2	x	100	2	x	100	2	x	5 weeks
13	3	TSB	0	x	x	0	x	x	0	x	x	0	x	x	1 week
14	1	TSB Liquid + MCs	**10000**	**x**	**x**	**5000**	**x**	**x**	1000	x	x	**1000**	**x**	**x**	5 weeks
14	2	TSA	**10000**	**0**	**x**	**5000**	**0**	**x**	1000	1	x	**1000**	**0**	**x**	5 weeks
14	3	TSB	**0**	**x**	**x**	**0**	**x**	**x**	0	x	x	**0**	**x**	**x**	1 week

Table 3.7: Fungal Culturing Conditions and Results

Notes: Same as for Table 3.6.

Exp #	Step #	Growth Medium	Diesel Fuel Conc (ppm)	Potential TPH Degraders Isolated	Cells/g based on Plate Counts	Naphth-alene Conc (ppm)	Potential PAH Degraders Isolated	cells/g based on Plate counts	PCB 1 Conc (ppb)	Potential PCB Degraders Isolated	Cells/g based on Plate Counts	DBZ Conc (ppb)	Potential Dioxin Degraders Isolated	Cells/g based on Plate Counts	Total Incuba-tion Time
1	1	CFMM Plate + MCs	*100*	*x*	*0*	*50*	*x*	*0*	*10*	*x*	*0*	*10*	*x*	*0*	5 weeks
1	2	YM Liquid	*0*	*0*	*x*	*0*	*0*	*x*	*0*	*0*	*x*	*0*	*0*	*x*	1 week
2	1	CFMM Plate + MCs	1000	x	800	500	x	1100	*100*	*x*	*0*	*100*	*x*	*0*	5 weeks
2	2	YM Liquid	0	1	x	0	1	x	*0*	*0*	*x*	*0*	*0*	*x*	1 week
3	1	CFMM Plate + MCs	10000	x	1200	5000	x	700	*1000*	*x*	*0*	1000	x	600	5 weeks
3	2	TSB	0	1	x	0	1	x	*0*	*0*	*x*	0	1	x	1 week
4	1	CFMM Plate + MCs	x	x	x	x	x	x	100000	x	1400	100000	x	1200	5 weeks
4	2	YM Liquid	x	x	x	x	x	x	0	2	x	0	2	x	1 week
5	1	CFMM Plate + MCs	*50*	*x*	*0*	*10*	*x*	*0*	x	x	x	x	x	x	5 weeks
5	2	TSB	*0*	*0*	*x*	*0*	*0*	*x*	x	x	x	x	x	x	1 week

Table 3.7 Continued…

Exp #	Step #	Medium	Diesel Fuel Conc (ppm)	Potential TPH Degraders Isolated	Cells/g based on Plate Counts	Naph-thalene Conc (ppm)	Potential PAH Degraders Isolated	Cells/g based on Plate Counts	PCB 1 Conc (ppb)	Potential PCB Degraders Isolated	Cells/g based on Plate Counts	DBZ Conc (ppb)	Potential Dioxin Degraders Isolated	Cells/g based on Plate Counts	Total Incub-ation Time
6	1	CFMM Liquid + MCs	*100*	*x*	*x*	*50*	*x*	*x*	*10*	*x*	*x*	*10*	*x*	*x*	5 weeks
6	2	CFMM Plates + MCs	*100*	*0*	*x*	*50*	*0*	*x*	*10*	*0*	*x*	*10*	*0*	*x*	5 weeks
6	3	YM Liquid	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
7	1	CFMM Liquid + MCs	*1000*	*x*	*x*	*500*	*x*	*x*	*100*	*x*	*x*	*100*	*x*	*x*	5 weeks
7	2	CFMM Plates + MCs	*1000*	*0*	*x*	*500*	*0*	*x*	*100*	*0*	*x*	*100*	*0*	*x*	5 weeks
7	3	YM Liquid	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
8	1	CFMM Liquid + MCs	*10000*	*x*	*x*	*5000*	*x*	*x*	*1000*	*x*	*x*	*1000*	*x*	*x*	5 weeks
8	2	CFMM Plates+ MCs	*10000*	*0*	*x*	*5000*	*0*	*x*	*1000*	*0*	*x*	*1000*	*0*	*x*	5 weeks
8	3	YM Liquid	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
9	1	CFMM Liquid + MCs	*100*	*x*	*x*	*50*	*x*	*x*	*10*	*x*	*x*	*10*	*x*	*x*	5 weeks
9	2	YM + MCs	*100*	*0*	*x*	*50*	*0*	*x*	*10*	*0*	*x*	*10*	*0*	*x*	5 weeks
9	3	YM Liquid	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week

Table 3.7 Continued…

Exp #	Step #	Medium	Diesel Fuel Conc (ppm)	Potential TPH Degraders Isolated	Cells/g based on Plate Counts	Naphth-alene Conc (ppm)	Potential PAH Degraders Isolated	Cells/g based on Plate Counts	PCB 1 Conc (ppb)	Potential PCB Degraders Isolated	Cells/g based on Plate Counts	DBZ Conc (ppb)	Potential Dioxin Degraders Isolated	Cells/g based on Plate Counts	Total Incub-ation Time
10	1	CFMM Liquid + MCs	*1000*	*x*	*x*	*500*	*x*	*x*	*100*	*x*	*x*	*100*	*x*	*x*	5 weeks
10	2	YM + MCs	*1000*	*0*	*x*	*500*	*0*	*x*	*100*	*0*	*x*	*100*	*0*	*x*	5 weeks
10	3	YM Liquid	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
11	1	CFMM Liquid + MCs	*10000*	*x*	*x*	*5000*	*x*	*x*	*1000*	*x*	*x*	*1000*	*x*	*x*	5 weeks
11	2	YM + MCs	*10000*	*0*	*x*	*5000*	*0*	*x*	*1000*	*0*	*x*	*1000*	*0*	*x*	5 weeks
11	3	YM Liquid	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
12	1	YM Liquid + MCs	*100*	*x*	*x*	*50*	*x*	*x*	*10*	*x*	*x*	*10*	*x*	*x*	5 weeks
12	2	YM Plate	*100*	*0*	*x*	*50*	*0*	*x*	*10*	*0*	*x*	*10*	*0*	*x*	5 weeks
12	3	YM Liquid	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week
13	1	YM Liquid + MCs	*1000*	*x*	*x*	*500*	*x*	*x*	*100*	*x*	*x*	*100*	*x*	*x*	5 weeks
13	2	YM Plate	*1000*	*0*	*x*	*500*	*0*	*x*	*100*	*0*	*x*	*100*	*0*	*x*	5 weeks
13	3	YM Liquid	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week

Table 3.7 Continued…

Exp #	Step #	Medium	Diesel Fuel Conc (ppm)	Potential TPH Degraders Isolated	Cells/g based on Plate Counts	Naphth-alene Conc (ppm)	Potential PAH Degraders Isolated	Cells/g based on Plate Counts	PCB 1 Conc (ppb)	Potential PCB Degraders Isolated	Cells/g based on Plate Counts	DBZ Conc (ppb)	Potential Dioxin Degraders Isolated	Cells/g based on Plate Counts	Total Incub-ation Time
14	1	YM Liquid + MCs	*10000*	*x*	*x*	*5000*	*x*	*x*	*1000*	*x*	*x*	*1000*	*x*	*x*	5 weeks
14	2	YM Plate	*10000*	*0*	*x*	*5000*	*0*	*x*	*1000*	*0*	*x*	*1000*	*0*	*x*	5 weeks
14	3	YM Liquid	*0*	*x*	*x*	*500*	*x*	*x*	*0*	*x*	*x*	*0*	*x*	*x*	1 week

Isolated bacteria and fungi were identified using 16S sequencing for bacteria and ITS sequencing for fungi. PCR was conducted using both fungal and bacterial primers to ensure that they would be sequenced and identified correctly. This yielded 21 unique organisms, including 14 bacteria and 7 fungi shown in Table 3.8. This included 3 strains of the fungi *Phanerochaete chrysosporium* and 6 strains of the bacteria *Pseudomonas*.

Table 3.8: Summary of Isolated Organisms

Contaminant Class	Petroleum Hydrocarbons	PAHs	PCBs	Dioxin
Bacteria Isolated	10	8	9	9
Fungi Isolated	2	2	2	3

Table 3.9 shows each of the isolated microorganisms and their potential for biodegrading the COIs based on a literature review. Ten of the bacteria and 3 of the fungi isolated are known degraders of the COIs or come from a genus that contains known degraders of the COIs. “Spiked model compound” is the COI that was used to isolate that microbe. If a microbe has been reported to biodegrade the COI that it was isolated with so, it was noted in the “Known to degrade COI isolated with?” column in Table 3.9. “Query cover” indicates the percent of the query sequence that overlaps with the subject sequence. The indent specifies the percent of the subject sequence that overlaps at the beginning of the sequence. It indicates how much sequence could have been lost due to where the primer is located on the gene. E value is a measure of random background noise. It describes the hits one can expect to see by chance when searching a database of a specific size. The closer and E value is to zero the more significant the match is. BLAST HIT indicates the top result of the query in the NCBI database. The query cover values were all 98% or higher for complete sequences, indicating that they are good matches to the database sequences. The indent percentages were all above 80%, and most were above 95%. This indicates that most of the sequence was replicated during PCR, leading to more accurate matches in the database. The E values were all zero except in 6 cases. In all 6 of the exceptions the E value was extremely low, indicating that all isolates had significant matches.

Table 3.9 also shows TRFLP fragment lengths expected for the sequences identified for the isolated bacteria and fungi. TRFLP uses restriction enzymes to cut DNA extracted from microbes into fragments. These restriction enzymes only cut at very specific DNA sequences. Since the 16S region of bacteria DNA and ITS regions of fungi DNA are highly variable these cuts will occur in different places for different organisms. The cuts result in fragments of DNA, whose length is specific to that microbe. However, multiple microbes (especially similar or related microbes) can have a similar 16S or ITS sequence,

resulting in similar or identical fragment lengths. Only DpnII-cut sites were looked for in bacteria sequences and only HaeIII cut sites for fungi. This is because the TRFLP data only had DpnII digests for bacteria and only HaeIII digests for fungi. The results of the comparison between the isolated microbes predicted TRFLP patterns and the actual fragment data are discussed below in Section 3.2.3.

Many of the species identified have not been reported as biodegraders of the COIs that they were isolated on. This does not necessarily mean that they are not biodegraders. Because all of these bacteria come from genera that contain biodegraders of the COIs it is possible that they too contain these genes and just have not been studied yet. For example, bacteria of the genus *Pseudomonas* were isolated 17 times on every COI except PAHs. Of these 17 there were 6 different BLAST hits, suggesting that they are distinct strains or species. Although none of the specific strains of *Pseudomonas* isolated are known biodegraders of the COIs that they were isolated on, *Pseudomonas* are very common soil bacteria with many different strains and a broad range of growth substrates (Juteau et al. 1999). Various *Pseudomonas* species biodegrade petroleum hydrocarbons (Das and Chandran 2011), PCBs (Hong et al. 2004), and dioxins (Hong et al. 2004). It is possible that some of the strains isolated have the capacity to biodegrade the COIs they were isolated on, but they just have not been studied and reported on.

Similarly for fungi, there were a few strains isolated that matched published degraders of the COI used to isolate them and two strains not known to degrade the COIs. The species known to degrade the COIs were *Aspergillus fumigatus* and *Phanerochaete chrysosporium*. Tigini et al. (2009) showed that certain strains of *Aspergillus fumigatus* have the capability to biodegrade PCBs with the addition of glucose. *Phanerochaete chrysosporium* has been shown to biodegrade PAHs (Bumpus 1989) as well as petroleum hydrocarbons (Pérez-Armendáriz et al. 2012), PCBs (Fernández-Sánchez et al. 2001), and dioxins (Hammel, Kalyanaraman, and Kirk 1986). The two isolates not matching known degraders were *Phanerochaete* species. However, similar to the case with bacteria, it is possible that these isolates have biodegrading capabilities, which have not yet been published.

Although these culturing experiments indicate there are microbes growing in the soil of the site with the capacity to biodegrade model chemicals similar to the COIs, it is important to remember that these are idealized conditions. The model chemicals selected are significantly easier to biodegrade than the COIs themselves. Also, complex interactions in the soil environment could affect the ability of these microorganisms to biodegrade the COIs.

Table 3.9: Cultured microorganisms identified using DNA sequencing and compared to microorganisms reported in the literature to biodegrade the COIs

Sample Name	Spiked Model Compound	Sample Origin	Sequence Length	BLAST HIT	Indent	Query Cover	E value	*DpnII* Fragment Length	*HaeIII* Fragment Length	Known to Degrade COI Isolated with?	Reference
D4	N/A	*S. wittichii* control	1346	*Sphingomonas wittichii*	99%	100%	0	129	N/A	N/A	T. R. Miller et al. 2010
D3	N/A	*P. putida* KF715 control	1371	*Pseudomonas sp*	99%	100%	0	181	N/A	N/A	Hayase, Taira, and Furukawa 1990
G2	N/A	*P. naphthalenovorans* control	1402	*Paenibacillus sp*	95%	98%	0	210	N/A	N/A	Daane et al. 2002
D2	N/A	*R. rhodochrous* control	1436	*Rhodocoicus pyridinivorans*	98%	99%	0	514	N/A	N/A	Sorkhoh et al. 1990
G1	N/A	*P. chrysosporium* I control	653	*Phanerochaete chrysosporium*	96%	100%	0	N/A	574	N/A	Fernández-Sánchez et al. 2001; Fernández-Luqueño et al. 2011; Pérez-Armendáriz et al. 2012; Hammel, Kalyanaraman, and Kirk 1986; Hammel 1992
I5	N/A	*P. chrysosporium* PR control	781	*Phanerochaete chrysosporium strain KCTC 6728*	99%	100%	0	N/A	58	N/A	N/A

Table 3.9 Continued…

Sample Name	Spiked Model Compound	Sample Origin	Sequence Length	BLAST HIT	Indent	Query Cover	E value	*DpnII* Fragment Length	*HAEIII* Fragment Length	Known to Degrade COI Isolated with?	Reference
D8	#2 Diesel Fuel	T10	1366	*Pseudomonas sp. RKS7-1*	99%	100%	0	160	N/A	Yes by spp.	Das and Chandran, 2011
A8	#2 Diesel Fuel	T05	1370	*Pseudomonas sp. RKS7-1*	99%	100%	0	181	N/A	Yes by spp.	Das and Chandran, 2011
J8	#2 Diesel Fuel	T03	1059	*Pseudomonas sp*	94%	99%	0	191	N/A	Yes	Das and Chandran, 2011
F5-reverse	#2 Diesel Fuel	T01	620	*Streptomyces nodosus*	83%	98%	2.00E-151	541	N/A	No	N/A
K1	#2 Diesel Fuel	T01	620	*Streptomyces nodosus*	83%	98%	2.00E-151	541	N/A	No	N/A
K2	#2 Diesel Fuel	T01	620	*Streptomyces nodosus*	83%	98%	2.00E-151	541	N/A	No	N/A
C1	#2 Diesel Fuel	T04	1363	*Streptomyces flaveolus*	99%	100%	0	551	N/A	No	N/A
F1-forward	#2 Diesel Fuel	T07	1322	*Streptomyces flaveolus*	99%	100%	0	551	N/A	No	N/A

Table 3.9 Continued…

Sample Name	Spiked Model Compound	Sample Origin	Sequence Length	BLAST HIT	Indent	Query Cover	E value	*DpnII* Fragment Length	*HAEIII* Fragment Length	Known to Degrade COI Isolated with?	Reference
J1	#2 Diesel Fuel	T08	1363	*Streptomyces flaveolus*	99%	100%	0	551	N/A	No	N/A
J2	#2 Diesel Fuel	T07	1363	*Streptomyces flaveolus*	99%	100%	0	551	N/A	No	N/A
I7-forward	#2 Diesel Fuel	T01	647	*Phanerochaete chrysosporium strain KCTC 6728*	92%	99%	0	N/A	56	No	N/A
I7-reverse	#2 Diesel Fuel	T01	598	*Uncultured Phanerochaete isolate*	83%	100%	1.00E-141	N/A	500	Yes	Yateem et al., 1998
J6	#2 Diesel Fuel	T01	598	*Uncultured Phanerochaete isolate*	83%	100%	1.00E-141	N/A	500	Yes	Yateem et al., 1998
B1	Naphthalene	T10	1368	*Arthrobacter oxydans*	100%	100%	0	97	N/A	Yes	Kallimanis et al., 2009
J5	Naphthalene	T10	1368	*Arthrobacter oxydans*	100%	100%	0	97	N/A	Yes	Kallimanis et al., 2009
J7	Naphthalene	T08	1368	*Arthrobacter oxydans*	100%	100%	0	97	N/A	Yes	Kallimanis et al., 2009
F4	Naphthalene	T09	660	*Streptomyces viridochromogenes*	92%	100%	0	109	N/A	Yes by spp.	Sutherland et al., 1990
E1	Naphthalene	T01	1368	*Arthrobacter oxydans*	100%	100%	0	118	N/A	Yes	Kallimanis et al., 2009

Table 3.9 Continued…

Sample Name	Spiked Model Compound	Sample Origin	Sequence Length	BLAST HIT	Indent	Query Cover	E value	*DpnII* Fragment Length	*HaeIII* Fragment Length	Known to Degrade COI Isolated with?	Reference
B7	Naphthalene	T02	1403	*Micromonospora sp.*	99%	100%	0	163	N/A	Yes in Consort.	Janbandhu and Fulekar, 2011
E7	Naphthalene	T04	1355	*Micromonospora chokoriensis*	99%	100%	0	1189	N/A	Yes, spp. in Consort.	Janbandhu and Fulekar, 2011
I8	Naphthalene	T04	1355	*Micromonospora chokoriensis*	99%	100%	0	1189	N/A	Yes, spp. in Consort.	Janbandhu and Fulekar, 2011
F8-forward	Naphthalene	T07	687	*Phanerochaete chrysosporium*	91%	79%	0	N/A	291	Yes	Bumpus, 1989
I4	Naphthalene	T09	617	*Aspergillus fumigatiaffinis*	99%	98%	0	N/A	85	Yes by spp.	Varanasi, *pg 54*
B4-reverse	PCB #1	P10	364	*Pantoea agglomerans*	93%	100%	2.00E-149	51	N/A	No	N/A
E4-reverse	PCB #1	P10	403	*Pantoea agglomerans*	97%	99%	0	51	N/A	No	N/A
B2	PCB #1	P01	1352	*Pseudomonas fluorescens*	99%	100%	0	180	N/A	Yes by spp.	Du et al., (2001)
B4-forward	PCB #1	P10	1047	*Pseudomonas sp*	94%	99%	0	180	N/A	Yes	Yong-lei et al. 2011
E2	PCB #1	P01	1344	*Pseudomonas fluorescens*	99%	100%	0	180	N/A	Yes by spp.	Du et al., (2001)

Table 3.9 Continued…

Sample Name	Spiked Model Compound	Sample Origin	Sequence Length	BLAST HIT	Indent	Query Cover	E value	*DpnII* Fragment Length	*HaeIII* Fragment Length	Known to Degrade COI Isolated with?	Reference
J4	PCB #1	P10	1047	*Pseudomonas sp*	94%	99%	0	180	N/A	Yes	Yong-lei et al. 2011
E4-forward	PCB #1	P10	1059	*Pseudomonas sp*	94%	99%	0	191	N/A	Yes	Yong-lei et al. 2011
E3	PCB #1	P03	1376	*Pseudomonas sp*	96%	99%	0	196	N/A	Yes	Yong-lei et al. 2011
B3	PCB #1	P03	1374	*Pseudomonas sp*	93%	99%	0	1073	N/A	Yes	Yong-lei et al. 2011
H2-reverse	PCB #1	P01	578	*Aspergillus fumigatiaffinis*	90%	95%	0	N/A	154	Yes by spp.	Tigini et al., 2009
I3	PCB #1	P09	635	*Aspergillus fumigatus*	99%	99%	0	N/A	64	Yes	Tigini et al., 2009
B8	DBZ	D07	1376	*Variovorax paradoxus*	99%	100%	0	91	N/A	Yes, dechlorinated	Kaiya et al., 2012
E8	DBZ	D10	1378	*Variovorax paradoxus*	99%	100%	0	152	N/A	Yes, dechlorinated	Kaiya et al., 2012
J3	DBZ	D03	1378	*Variovorax paradoxus*	99%	100%	0	152	N/A	Yes, dechlorinated	Kaiya et al., 2012
A5	DBZ	D07	1370	*Pseudomonas koreensis*	99%	100%	0	181	N/A	Yes by spp.	Du et al., (2001)

Table 3.9 Continued…

Sample Name	Spiked Model Compound	Sample Origin	Sequence Length	BLAST HIT	Indent	Query Cover	E value	*DpnII* Fragment Length	*HaeIII* Fragment Length	Known to Degrade COI Isolated with?	Reference
A7	DBZ	D06	1367	*Pseudomonas fluorescens*	99%	100%	0	181	N/A	Yes by spp.	Du et al., (2001)
D5	DBZ	D07	1370	*Pseudomonas sp*	99%	100%	0	181	N/A	Yes	Hong et al., (2004)
D7	DBZ	D06	1372	*Pseudomonas fluorescens*	99%	99%	0	185	N/A	Yes by spp.	Du et al., (2001)
K3	DBZ	D06	1372	*Pseudomonas fluorescens*	99%	99%	0	185	N/A	Yes by spp.	Du et al., (2001)
A6	DBZ	D05	1383	*Pseudomonas sp. b17*	99%	100%	0	194	N/A	Yes by spp.	Hong et al., (2004)
D6	DBZ	D09	1394	*Pseudomonas fluorescens Pf0-1*	99%	99%	0	194	N/A	Yes by spp.	Du et al., (2001)
H5	DBZ	D05	641	*Aspergillus fumigatiaffinis*	99%	97%	0	N/A	86	No	N/A
H6	DBZ	D08	691	*Gongronella butleri*	99%	94%	0	N/A	460	No	N/A
I2	DBZ	D07	622	*Penicillium sp.*	86%	98%	0	N/A	69	Yes	Shetty, Zheng, and Levin, 1999

3.2.3. Results of TRFLP Analysis: Bacteria

Raw TRFLP data can be presented as a chromatogram with each peak representing a different fragment length associated with different microorganisms (see Figure 3.3). The height of each peak indicates the relative abundance of that fragment in that sample. The blue lines are the fragments from the DNA sample. The red lines are fragments from a standard solution. These standards are used for QA/QC purposes. For both sets of fragment data (bacterial and fungi), fragment data from each of the 30 samples were processed and compiled into an excel spreadsheet.

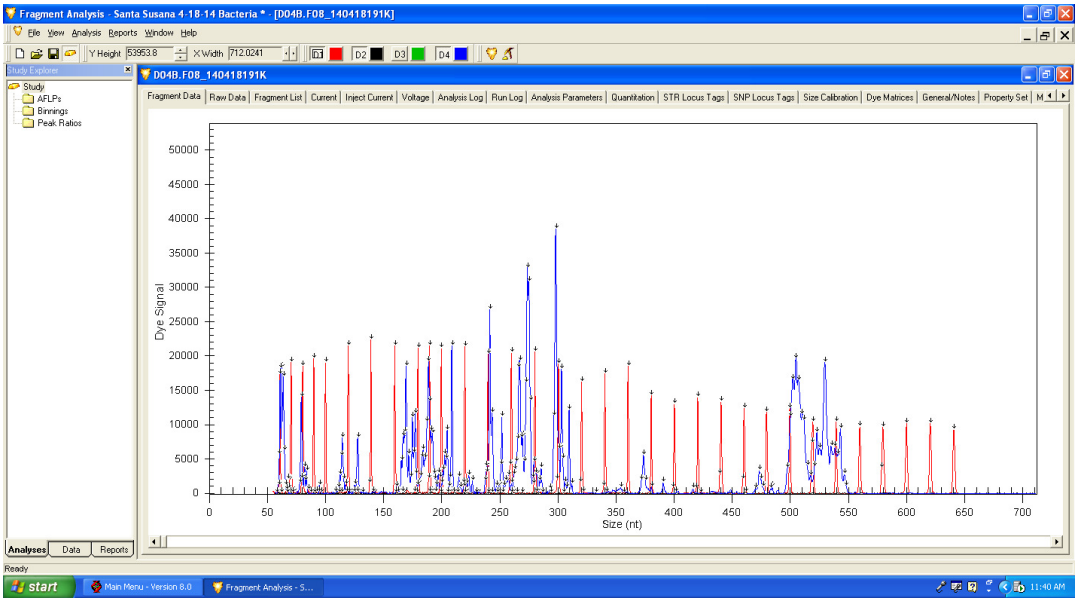

Figure 3.3: An example of a TRFLP chromatogram

The program Primer 5 was used to produce a similarity matrix using the processed fragment analysis for bacteria. A similarity matrix is a matrix of values that represent how similar each data point is to each other data point. This matrix is used to describe the relative difference between each sample. The magnitude of these differences show how similar or dissimilar microbial communities in these soil samples are. These differences were visualized using multidimensional scaling (MDS). Because the placement of the data points is based on relative similarity to other data points, there are no specific parameters or scales associated with the axes on an MDS plot. Points are placed based on the relative distance between their respective fragment data, so the chart can be rotated in any direction without changing this distance (Borg and Groenen 1997). For this reason axis labels are normally left off. Factors can then be added to label the data points of the MDS plot in different ways. For this analysis the factors chosen were COI series, soil type (e.g. sandy loam vs. clay), location, presence of TPH, presence of PAHs, presence of PCBs, and presence of dioxin. COI series was defined by which sample set the sample

came from (i.e. TPH/PAH, dioxin, or PCBs). Table 3.10 shows these factors for each sample. Presence of TPH was determined by a threshold of 350 ppm. Presence of PAHs was determined by a threshold of 2.5 ppm. Presence of PCBs was determined by a threshold of 450 ppb. Presence of dioxin was determined by a threshold of 5 ppb. Table 3.1 has the specific concentrations of each COI for each sample. Location was determined by the relative positions of the sample locations shown in Figure 3.1. The MDS scatter plot for bacterial data were labeled using different factors to reveal possible patterns.

Table 3.10: Factors used in MDS Analysis of TRFLP Data

Sample	COI Series	Soil Type	Location	TPH	PAHs	PCBs	Dioxins
T01	TPH/PAHs	Sandy Loam	Central	Y	Y	Y	N
T02	TPH/PAHs	Sandy Loam	Central	Y	Y	N	N
T03	TPH/PAHs	Sandy Loam	Central	N	Y	N	Y
T04	TPH/PAHs	Sand	Central	Y	Y	N	N
T05	TPH/PAHs	Silt	South	N	Y	N	Y
T06	TPH/PAHs	Silt Loam	North	Y	Y	N	N
T07	TPH/PAHs	Sandy Loam	Central	N	Y	N	N
T08	TPH/PAHs	Clay	South	N	Y	N	N
T09	TPH/PAHs	Sandy Loam	Central	Y	N	N	N
T10	TPH/PAHs	Sandy Loam	Central	Y	Y	N	N
P01	PCBs	Sandy Loam	North	Y	N	Y	Y
P02	PCBs	Sandy Loam	North	N	N	Y	N
P03	PCBs	Clay	South	-	-	Y	-
P04	PCBs	Silt	North	Y	Y	Y	N
P05	PCBs	Silt Loam	North	Y	N	Y	N
P06	PCBs	Silt Loam	Central	Y	Y	Y	N
P07	PCBs	Sandy Loam	South	-	-	Y	-
P08	PCBs	Clay	South	-	-	Y	-
P09	PCBs	Clay	South	-	-	Y	-
P10	PCBs	Clay	South	-	-	Y	-
D01	Dioxin	Clay Loam	Central	-	N	N	Y
D02	Dioxin	Silt	South	-	-	-	Y
D03	Dioxin	Silt	Central	N	Y	N	Y
D04	Dioxin	Silt Loam	Central	N	Y	N	Y
D05	Dioxin	Sandy Loam	North	Y	N	N	Y
D06	Dioxin	Sandy Loam	Central	-	-	N	Y
D07	Dioxin	Sand	Central	N	Y	N	Y
D08	Dioxin	Sandy Loam	North	N	N	N	Y
D09	Dioxin	Sandy Loam	South	N	Y	N	Y
D10	Dioxin	Sandy Loam	Central	N	Y	N	Y

An example of a scatter plot comparing the TRFLP patterns based on the factors in Table 3.10 is shown in Figure 3.4, and the remainder are shown in Appendix B. Figure 3.4 shows an MDS scatterplot for bacterial fragment data labeled with the COI of the sample set that each sample came from. The factors used for the other scatter plots (shown in Appendix B) were COI series, location, soil type, presence of TPH, presence of PAHs, presence of PCBs, and presence of dioxin. The stress value, which is a measure of how reliable the representation is, is shown in the upper right corner of each plot. A stress value of 0.25 or less means the data are well represented.

Primer 5 was again used to calculate the indices of multivariate dispersion between each label within each factor (Table 3.11). This index value must be between -0.05 and 0.05 to be significant. This determines if there is a significant difference between the microbial populations of two different labels based on a group of factors. For example, within the factor of COI series, the index value for T and P is -0.17. This indicates that there is not a significant difference between the 10 samples in the TPH/PAH set and the 10 samples in the PCB sample set. There was no significant correlation between any of these factors and the bacterial populations except in soil type. There was a significant difference in the microbial populations of samples that had sandy loam soil and those that had clay soil (and index value of -.024). The difference in microbial communities between samples that were above the 450 ppb threshold for PCBs compared to those below the threshold was nearly significant (index value of 0.06).

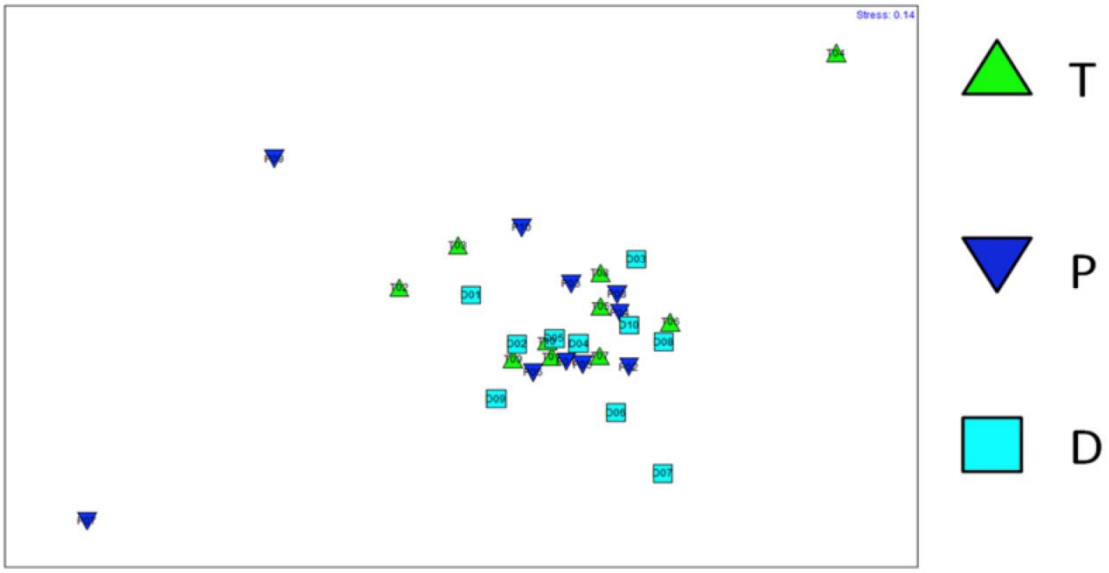

Figure 3.4: MDS from bacterial fragments using COI series as a factor. T is for the TPH/PAH sample set, P is for the PCB sample set, and D is for the dioxin sample set. The scatter of the points indicate no grouping of the microbial populations based on COI series.

Table 3.11: Indices of Multivariate Dispersion for each Factor: Bacteria

Factor	Two Groups Compared	Index of Multivariate Dispersion
COI series	TPH and PAHs, PCBs	-0.17
COI series	TPH and PAHs, Dioxins	0.161
COI series	PCBs, Dioxins	0.18
Location	Central, South	-0.138
Location	Central, North	0.699
Location	South, North	0.677
Soil Type	Sandy Loam, Sand	-0.802
Soil Type	Sandy Loam, Silt	0.308
Soil Type	Sandy Loam, Silty Loam	0.377
Soil Type	Sandy Loam, Clay	-0.024
Soil Type	Sand, Silt	1
Soil Type	Sand, Silty Loam	1
Soil Type	Sand, Clay	1
Soil Type	Silt, Silty Loam	0.167
Soil Type	Silt, Clay	-0.267
Soil Type	Silty Loam, Clay	-0.367
P/A of TPH	Yes, No	-0.069
P/A of PAHs	Yes, No	0.286
P/A of PCBs	Yes, No	0.06
P/A of Dioxins	Yes, No	-0.14

Legend: T = Sample from the TPH or PAHs sample sets, P = Sample from the PCBs sample set, D = Sample from the Dioxins sample set, C = Central, N = North, S = South, Y = Yes, N = No

Possible effects of contaminant concentration on the bacterial community were visualized by graphing the MDS plots using bubbles to denote the COI concentration of each sample, with bubble size proportional to the concentration of the contaminant being investigated in that analysis. For these bubble plots only individual sample sets of 10 were used in order minimize "noise" from the other 20 samples. The other samples are represented on the scatter plots as "0"s. They are important because they can show the similarity between the samples with bubbles (which are within that set of 10 soil samples) and the other soil samples (which are not in the set of 10). This was performed for all 4 COIs (Figure 3.5 for TPH, Figure 3.6 for PAHs, Figure 3.7 for PCBs, and Figure 3.8 for dioxin). Figure 3.7 represents the log scale PCB concentration in order to give the lower-concentration data more visibility. If there was a significant effect of COI concentration the high concentration samples would be grouped together away from the lower concentration samples. However, in this study the samples with high and low COI concentrations

were not grouped away from each other for any COIs. This indicates that the concentrations of COIs did not have any reproducible effect on the TRFLP pattern, and thus did not likely impact the microbial community population dynamics. Although the log scale PCB scatterplot show that most high concentrations are grouped together, they are not grouped away from neither the low concentration bubbles nor the soil samples from the other 2 sets of soil samples. This means that although the microbial communities in the PCB soil samples are similar, they are also similar to most of the other microbial communities in other soil samples with less PCBs.

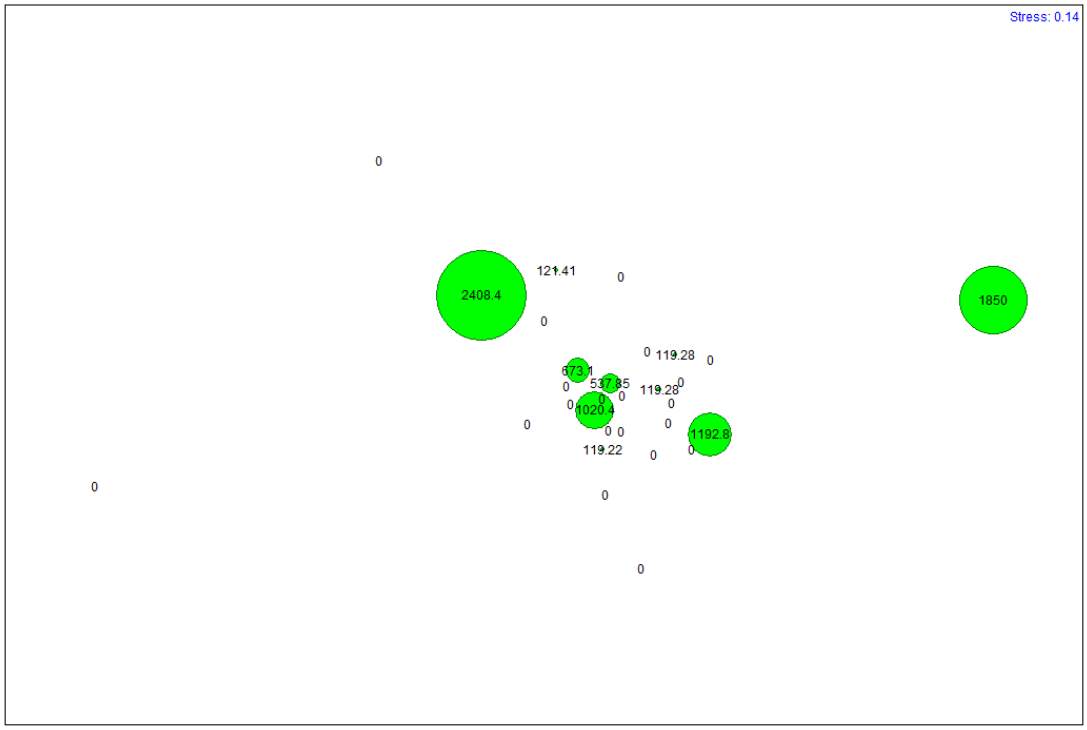

Figure 3.5: MDS from bacterial DNA fragments with bubble size based on TPH concentration

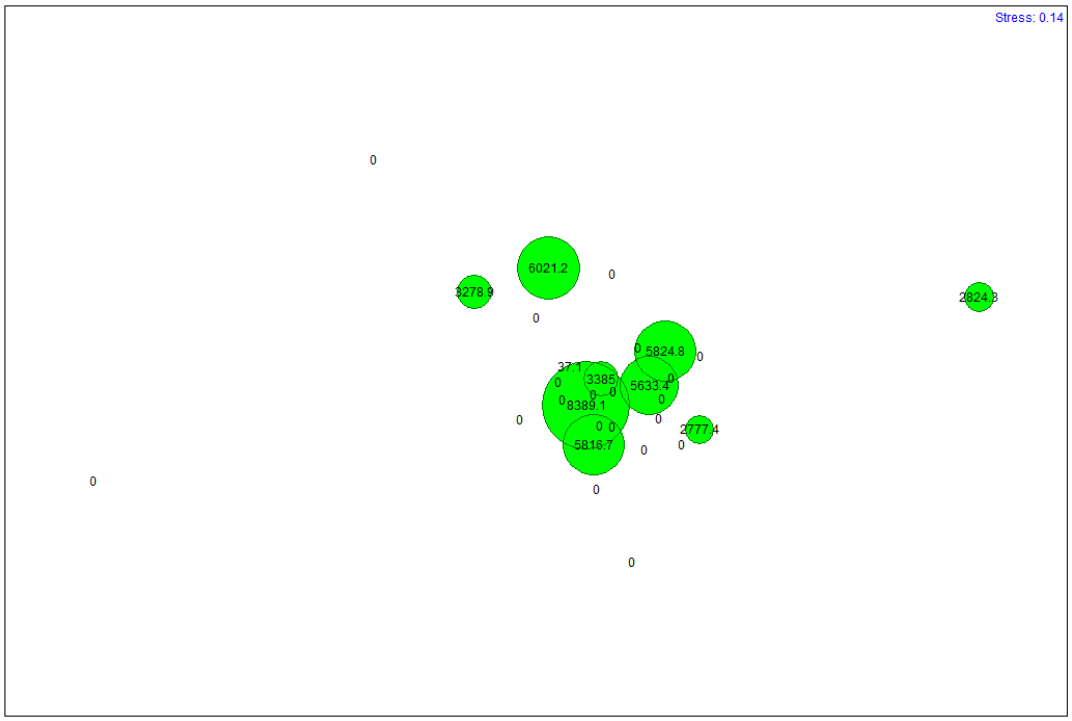

Figure 3.6: MDS from bacterial DNA fragments with bubble size based on PAH concentration.

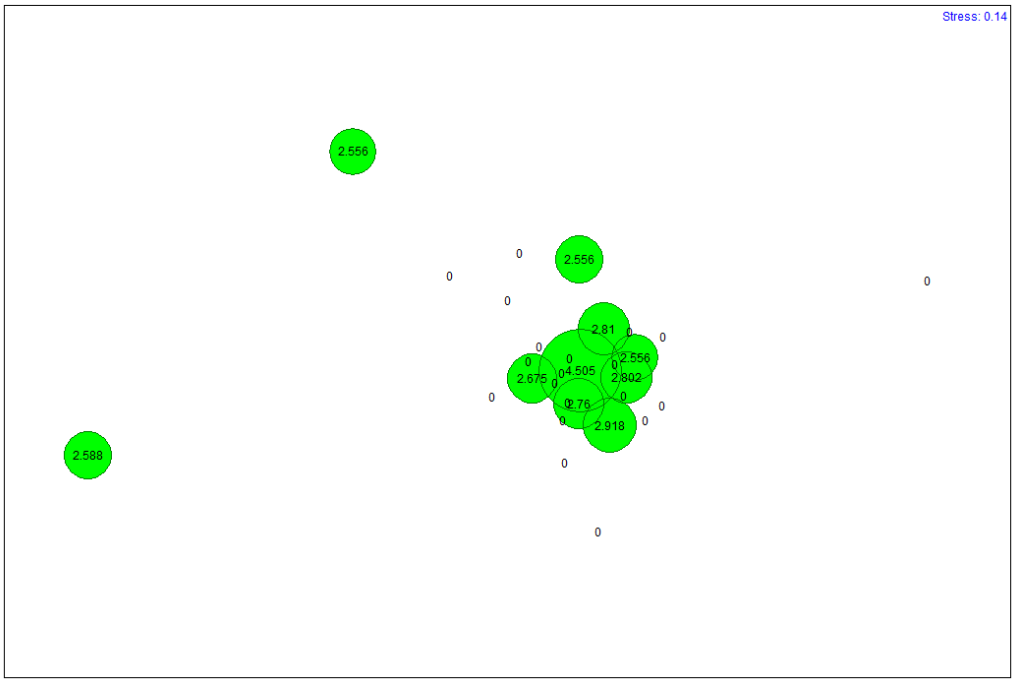

Figure 3.7: MDS from bacterial **DNA fragments with bubble size based on log of PCB concentrations**

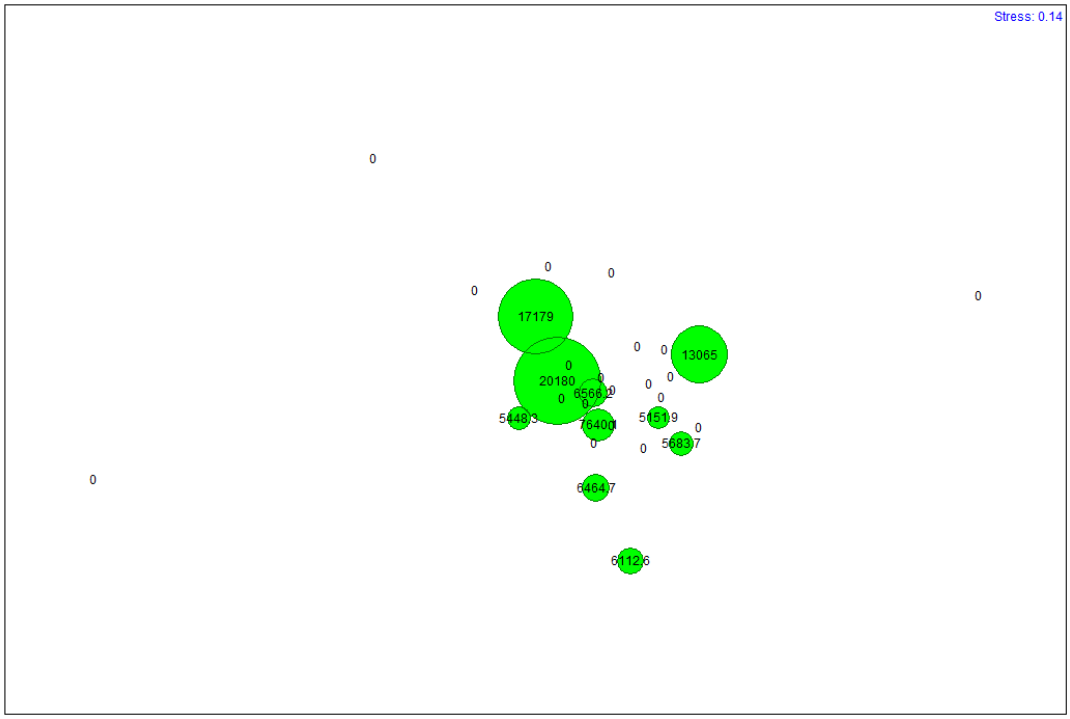

Figure 3.8: MDS from bacterial fragments with dioxin bubble size based on dioxin concentrations

3.2.4. Results of TRFLP Analysis: Fungi

TRFLP patterns of fungal species were analyzed in the same way as for the bacterial species as described above. The indices of multivariate dispersion are shown in Table 3.12. Again the factors examined were COI series, location, soil type, and presence of each of the COIs. The MDS scatter plots for each of these factors can be found in Appendix B. There was no significant correlation between any of these factors and the bacterial populations. Unlike with the bacterial data, even the soil type appeared to have no discernable effect on microbial population dynamics.

Table 3.12: Indices of Multivariate Dispersion for each Factor: Fungi

Bacteria/ Fungi	Factor	Two Groups Compared	Index of Multivariate Dispersion
Fungi	COI Series	TPH and PAHs, PCBs	-0.224
Fungi	COI Series	TPH and PAHs, Dioxins	0.235
Fungi	COI Series	PCBs, Dioxins	0.406
Fungi	Location	Central, South	-0.298
Fungi	Location	Central, North	0.298
Fungi	Location	South, North	0.466
Fungi	Soil Type	Sandy Loam, Sand	0.824
Fungi	Soil Type	Sandy Loam, Silt	-0.15
Fungi	Soil Type	Sandy Loam, Silty Loam	-0.136
Fungi	Soil Type	Sandy Loam, Clay	-0.756
Fungi	Soil Type	Sand, Silt	-0.667
Fungi	Soil Type	Sand, Silty Loam	-0.667
Fungi	Soil Type	Sand, Clay	-0.8
Fungi	Soil Type	Silt, Silty Loam	-0.167
Fungi	Soil Type	Silt, Clay	-0.767
Fungi	Soil Type	Silty Loam, Clay	-0.733
Fungi	P/A of TPH	Yes, No	0.192
Fungi	P/A of PAHs	Yes, No	-0.358
Fungi	P/A of PCBs	Yes, No	0.26
Fungi	P/A of Dioxins	Yes, No	0.143

Legend: T = Sample from the TPH or PAHs sample sets, P = Sample from the PCBs sample set, D = Sample from the Dioxins sample set, C = Central, N = North, S = South, Y = Yes, N = No

As was done for bacteria, possible effects of contaminant concentrations on the fungal community were visualized by adding bubbles to each data point that are proportional to the concentration of the contaminant being investigated in that analysis. This was performed for all 4 COIs (Figure 3.9 for TPH, Figure 3.10 for PAHs, Figure 3.11 for PCBs, and Figure 3.12 for dioxins). The bubbles in Figure 3.11 for PCBs represent the log of PCB concentration in order to provide visibility over the wide range of PCB concentrations. None of the COI concentrations had significant correlations to microbial populations based on these plots.

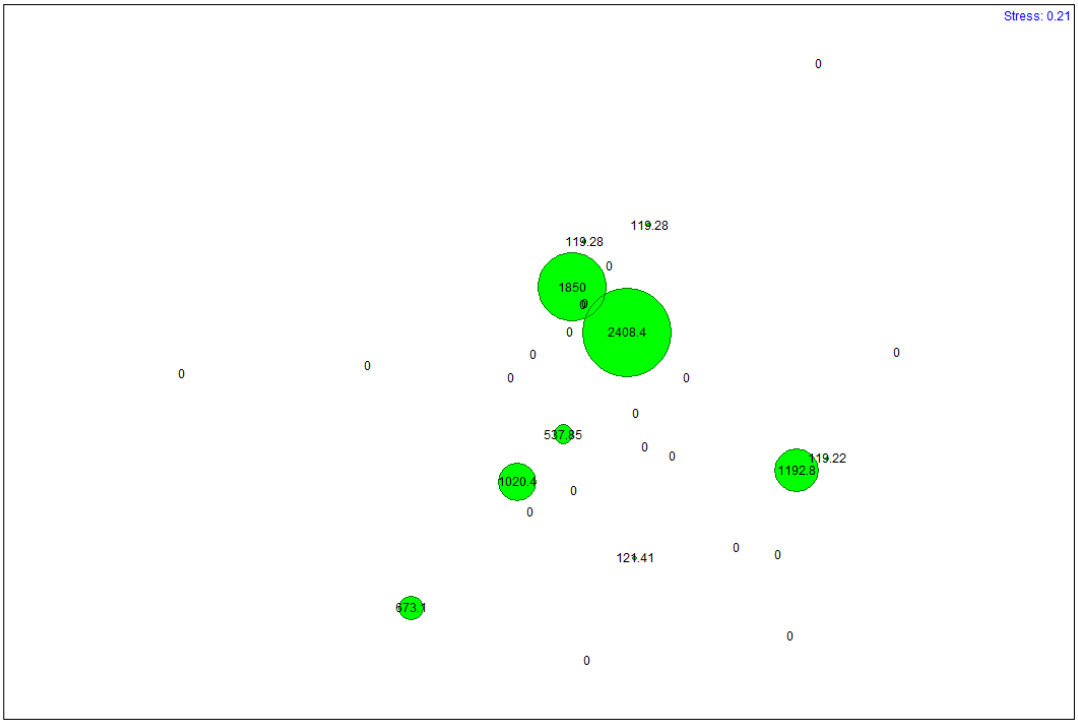

Figure 3.9: MDS of fungal TRFLP pattern with bubble size based on TPH concentration

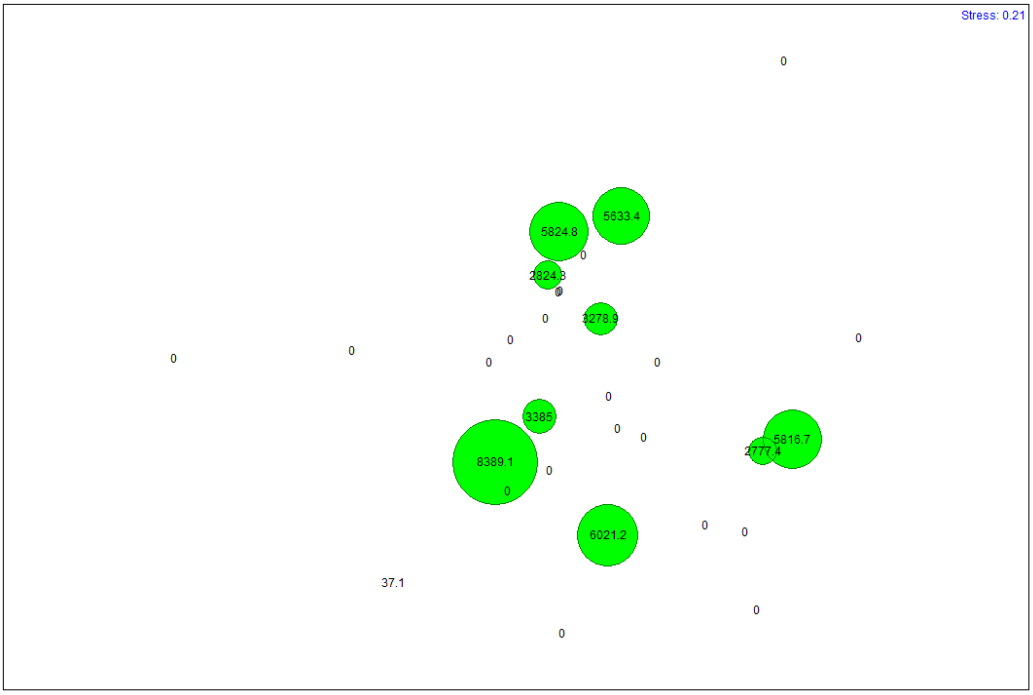

Figure 3.10: MDS of fungal TRFLP pattern with bubble size based on PAH concentration

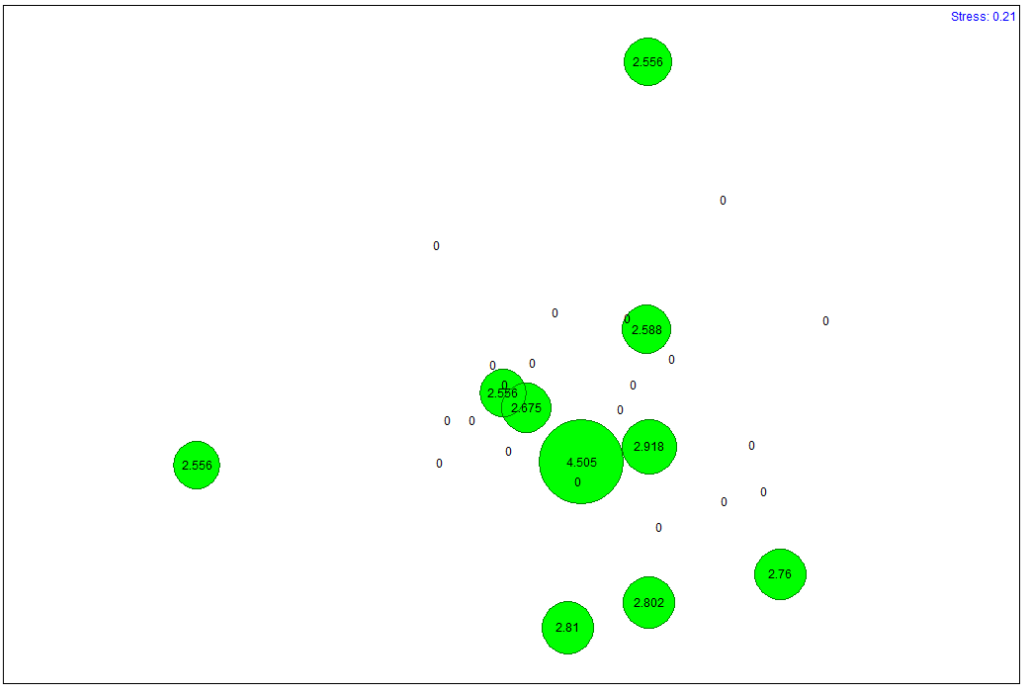

Figure 3.11: MDS from fungal TRFLP pattern with bubble size based on log PCB concentration

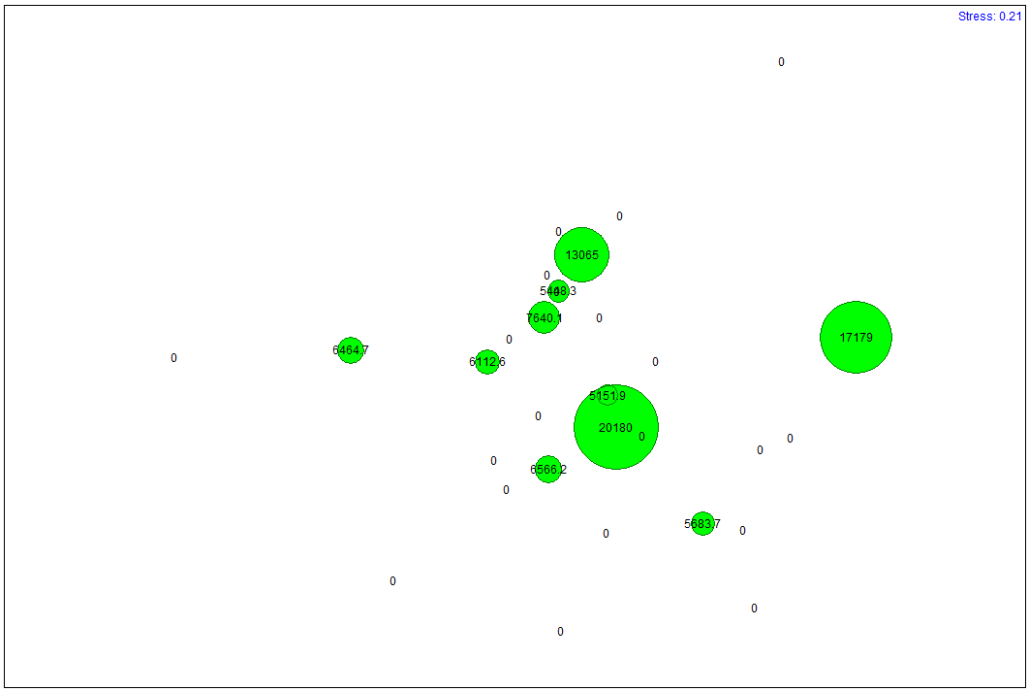

Figure 3.12: MDS from fungal TRFLP pattern with bubble size based on dioxin concentrations

3.3.5. TRFLP analysis of cultured microorganisms and control microorganisms

For each isolated bacteria or fungi which were potential degraders of the COIs the equivalent TRFLP fragment length was determined using the sequence data. These fragments were then searched for in the TRFLP fragment data. TRFLP patterns of the positive control microorganisms (model organisms) were also determined. For each isolated microbe, the COI it was isolated on, the TRFLP fragment length, the name of the microbe, the samples that contained its TRFLP peak, the relative abundance of this peak in the sample, if it is known to biodegrade the COI it was isolated on, and references are shown in Table 3.13. Sixteen out of the 21 isolates and control organisms had their fragment peak appear in at least one soil sample. The TRFLP peaks of all 6 positive control organisms were found in the fragment data as well.

Combining the TRFLP and culturing data reveals the distribution of these microbes across the site. Six of the isolates had their TRFLP peak appear in 5 or more of the 30 samples. Four of these were seen in 9 or more of the 30 samples. These microbes are making up a relatively small percentage of the total microbial population, meaning that other non-degraders make up the vast majority of the population. These non-degraders are most likely common soil microbes, which is why the TRFLP patterns between samples were so similar.

It is important to note that the presence of a TRFLP peak in a sample does not indicate that a microbe is definitely present because many microbes share the same fragment length. However,

in the case of the 22 organisms whose TRFLP peaks were reported and that were cultured directly from the soil it is likely that they do exist in site soil and that the peaks do represent them. The 19 reported degraders cover all 4 COIs and include both fungi and bacteria. The majority of these TRLFP peaks are between 1.5% and 5% of the total abundance of fragments assayed.

Table 3.13: TRFLP Fragment Analysis of Cultured Organisms

(* = measurement out of range; # = microbe was isolated multiple times from the same soil sample. The number in the brackets indicates the number of times it was isolated from that sample)

Bacteria /Fungi	Spiked Model Compound	BLAST HIT	*DpnII* Fragment Length	*HaeIII* Fragment Length	Samples that Contained Microbe Based on Culturing Experiments	Samples that Contained Microbe Based on TRFLP	Relative Abundance of Microbe in Soil Sample	Known to Degrade COI Isolated with?	Ref
Bacteria	N/A	*Rhodocoicus pyridinivorans*	514	N/A	*R. rhodochrous* control (petroleum hydrocarbons)	D06	2.0%	N/A	Sorkhoh et al. 1990
Bacteria	N/A	*Paenibacillus sp*	210	N/A	*P. naphthalenovorans* control (PAHs)	T01, T02, T07, P02, P05, P09, D01, D04, D05, D06	2.2%, 1.5%, 2.4%, 1.5%, 2.0%, 1.3%, 2.2%, 3.5%, 1.7%, 3.1%	N/A	Daane et al. 2002
Bacteria	N/A	*Pseudomonas sp*	181	N/A	*P. putida* KF715 control (PCBs)	P07, P09, D02,	5.3%, 3.4%, 1.6%	N/A	Hayase, Taira, and Furukawa 1990
Bacteria	N/A	*Sphingomonas wittichii*	129	N/A	*S. wittichii* control (Dioxin)	D01, D04	1.3%, 2.1%	N/A	T. R. Miller et al. 2010
Fungi	N/A	*Phanerochaete chrysosporium*	N/A	574	*P. chrysosporium* I control	P05, D08	8.8%, 2.1%	N/A	Fernández-Sánchez et al. 2001
Fungi	N/A	*Phanerochaete chrysosporium strain KCTC 6728*	N/A	58	*P. chrysosporium* PR control	T02, T03, T09, P02, P03, P07, P10, D02, D04, D05, D06, D08	2.3%, 5.4%, 5.9%, 3.1%, 4.1%, 1.4%, 18.7%, 1.5%, 1.5%, 1.3%, 5.3%, 3.4%	N/A	Fernández-Sánchez et al. 2001
Bacteria	Naphthalene	*Arthrobacter oxydans*	97	N/A	T01, T08, T10 (2)	P09	1.3%	Yes	Kallimanis et al., 2009
Bacteria	Naphthalene	*Micromonospora chokoriensis*	1189	N/A	T04 (2)	N/A*	N/A	Yes, spp. in Consort.	Janbandhu and Fulekar, 2011
Bacteria	Naphthalene	*Micromonospora sp.*	163	N/A	T02	P07, P09	2.6%, 2.7%	Yes in Consort.	Janbandhu and Fulekar, 2011

Table 3.13 Continued…

Bacteria /Fungi	Spiked Model Compound	BLAST HIT	*DpnII* Fragment Length	*HaeIII* Fragment Length	Samples that Contained Microbe Based on Culturing Experiments	Samples that Contained Microbe Based on TRFLP	Relative Abundance of Microbe in Soil Sample	Known to Degrade COI Isolated with?	Ref
Bacteria	PCB #1	*Pantoea agglomerans*	51	N/A	P10 (2)	N/A*	N/A	No	N/A
Bacteria	DBZ	*Pseudomonas fluorescens*	181	N/A	D06 (3), P01	P07, P09, D02,	5.3%, 3.4%, 1.6%	Yes by spp.	Du et al., (2001)
Bacteria	DBZ	*Pseudomonas fluorescens Pf0-1*	194	N/A	D09	T01, T06, P01, P04, P08, P10, D03, D06, D08	1.5%, 1.5%, 1.6%, 2.2%, 1.6%, 1.5%, 2.0%, 2.5%, 3.5%	Yes by spp.	Du et al., (2001)
Bacteria	DBZ	*Pseudomonas koreensis*	181	N/A	D07	P07, P09, D02,	5.3%, 3.4%, 1.6%	Yes by spp.	Du et al., (2001)
Bacteria	DBZ	*Pseudomonas sp*	181	N/A	D07, P03 (2), P10 (3), T03	P07, P09, D02,	5.3%, 3.4%, 1.6%	Yes	Hong et al., (2004)
Bacteria	DBZ	*Pseudomonas sp. b17*	194	N/A	D05	T01, T06, P01, P04, P08, P10, D03, D06, D08	1.5%, 1.5%, 1.6%, 2.2%, 1.6%, 1.5%, 2.0%, 2.5%, 3.5%	Yes by spp.	Hong et al., (2004)
Bacteria	#2 Diesel Fuel	*Pseudomonas sp. RKS7-1*	181	N/A	T05, T10	P07, P09, D02,	5.3%, 3.4%, 1.6%	Yes by spp.	Das and Chandran, 2011
Bacteria	#2 Diesel Fuel	*Streptomyces flaveolus*	551	N/A	T04, T07 (2), T08	none	N/A	No	N/A
Bacteria	#2 Diesel Fuel	*Streptomyces nodosus*	541	N/A	T01 (3)	T05, T06, T08, D06, D07, D08, D10	2.5%, 2.2%, 1.4%, 1.9%, 4.7%, 3.7%	No	N/A
Bacteria	Naphthalene	*Streptomyces viridochromogenes*	109	N/A	T09	none	N/A	Yes by spp.	Sutherland et al., 1990
Bacteria	DBZ	*Variovorax paradoxus*	152	N/A	D03, D07, D10	none	N/A	Yes, unchlorinated	Kaiya et al., 2012

Table 3.13 Continued…

Bacteria /Fungi	Spiked Model Compound	BLAST HIT	*DpnII* Fragment Length	*HaeIII* Fragment Length	Samples that Contained Microbe Based on Culturing Experiments	Samples that Contained Microbe Based on TRFLP	Relative Abundance of Microbe in Soil Sample	Known to Degrade COI Isolated with?	Ref
Fungi	DBZ	*Aspergillus fumigatiaffinis*	N/A	86	D05, P01, T09	T01, T02, T03, T04, T05, T06, T07, T08, T10, P01, P02, P03, P06, P07, P08, D01, D02, D03, D04, D05, D06, D07, D09, D10	8.2%, 5.2%, 34.0%, 4.5%, 2.0%, 1.7%, 1.4%, 3.2%, 4.1%, 3.5%, 33.5%, 4.7%, 4.7%, 7.2%, 3.7%, 1.3%, 11.5%, 2.1%, 12.7%, 5.0%, 4.5%, 6.8%, 3.3%, 10.7%	No	N/A
Fungi	PCB #1	*Aspergillus fumigatus*	N/A	64	P09	T05	3.6%	Yes	Tigini et al., 2009
Fungi	#2 Diesel Fuel	*Uncultured Phanerochaete isolate*	N/A	500	T01 (2)	none	N/A	Yes	Yateem et al., 1998
Fungi	DBZ	*Penicillium sp.*	N/A	69	D07	none	N/A	Yes	Shetty, Zheng, and Levin, 1999
Fungi	Naphthalene	*Phanerochaete chrysosporium*	N/A	291	T07	T06, T07	5.7%, 5.8%	Yes	Bumpus, 1989
Fungi	#2 Diesel Fuel	*Phanerochaete chrysosporium strain KCTC 6728*	N/A	56	T01	T05	1.4%	No	N/A
Fungi	DBZ	*Gongronella butleri*	N/A	460	D08	P04, D01	1.5%, 2.8%	No	N/A

3.2.6. Results of metagenomics assay

The taxonomic breakdown of the site soils, as determined by metagenomics sequencing at Sandia National Laboratory, is shown in Figure 3.13. More detailed taxonomic breakdowns for the five soil samples with the highest populations are shown in Appendix C. Attempts were made to correlate the taxonomic profiles with specific COI concentrations, but no significant correlations were identified. These results are similar to those for the TRFLP analysis which suggest that either specific bacteria and/or fungi associated with the COIs are either in low populations or distributed somewhat evenly across the site. More specific identification of specific species and gene targets associated with biodegradation is described below using qPCR.

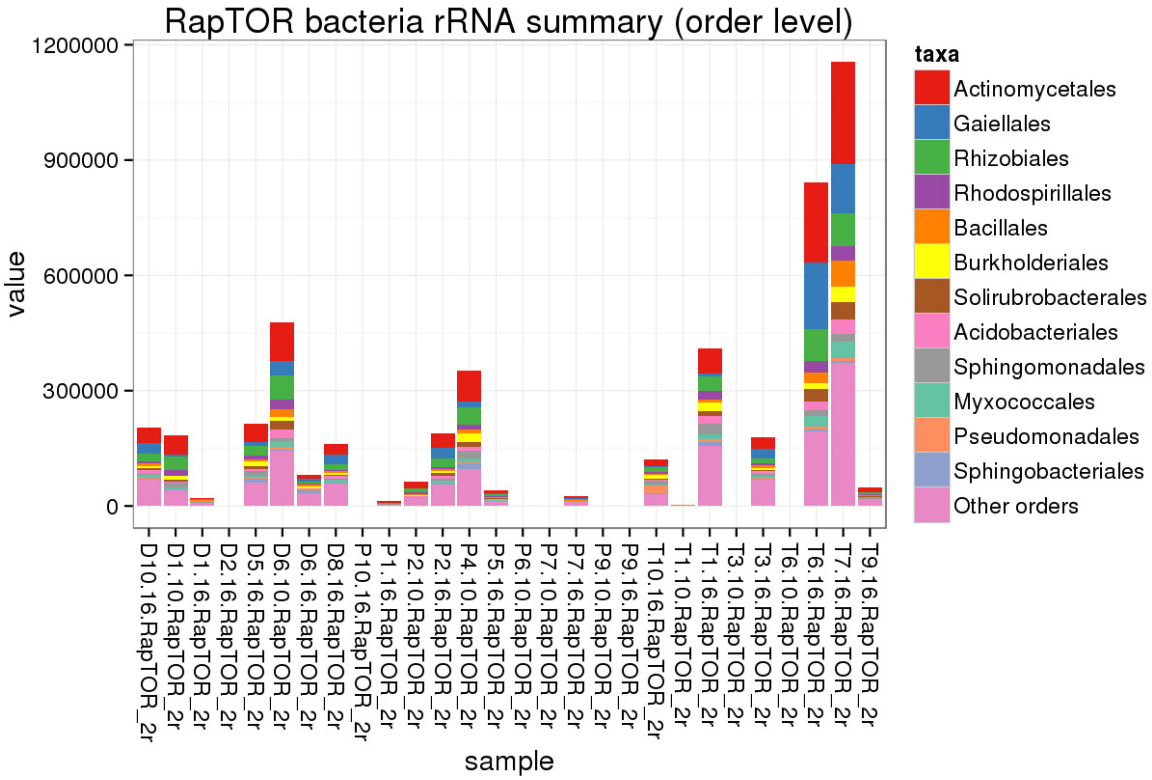

Figure 3.13 Metagenomic bacterial rRNA summary (order level) showing the bacterial taxa of 28 soil samples from the site (Data courtesy of Todd Lane, Kelly Williams and Kunal Poorey at Sandia National Laboratory)

3.2.7. Results of qPCR analysis of the soil microbial community

Results of the qPCR analysis are shown in Table 3.14 which gives the cell population associated with each gene target in units of number of cells per gram of soil. The qPCR analysis revealed several important things about the microbial community in the soil at the site, most notably that a significant population of microbes was found in both soil samples tested that are known to aerobically degrade petroleum hydrocarbons. The genes for aerobically breaking down toluene, biphenyl and phenol were detected in both samples. The qPCR analysis detected 5 of the 6 aerobic BTEX degradation targets in the soils. Four of these 6 were detected in both soil samples. The cell populations that contained these targets varied between $4.8x10^8$ to $4.9x10^4$ cells/g.

No anaerobic BTEX targets in either soil sample were detected with the exception of benzoyl coenzyme A (BCR), which is associated with anaerobic BTEX biodegradation. Sample 1 had 8.2 $x10^8$ cells/g of bacteria with BCR and Sample 2 had 7.1 $x10^7$ cells/g. BCR is a common intermediate that is formed in many pathways for anaerobic biodegradation of aromatic hydrocarbons. It is surprising that this gene was detected because the soil samples were expected to be aerobic. It is possible that the site soils are heterogeneous and that anaerobic microenvironments are present which could provide a habitat for these anaerobic microorganisms. No aerobic or anaerobic PAH biodegradation targets were detected.

Dehalococcoides spp., which is associated with reductive dechlorination of PCBs and dioxins (Fennell, 2004; Bunge and Lechner 2009; Bedard, Ritalahti, and Löffler 2007), was detected in Sample 1 (fresh soil from D03), but only in a small amount (Table 3.14). Again, the overall aerobic conditions in the site soils would not be expected to support the growth of such anaerobic bacteria, but there may be microenvironments which could harbor them. Since both *Dehalococcoides* and biphenyl dioxygenase were found in Sample 1, it is conceivable that microbes in that community are dechlorinating PCBs in anaerobic microenvironments and then breaking down the resulting biphenyl backbone. However, the very low population of *Dehalococcoides* detected suggests that this biodegradation mechanism would be expected to be insignificant.

Table 3.14 Summary of the QuantArray® Petro qPCR results

(highlighted numbers indicate detection of gene targets)

Analysis	Sample 1 (from D03) (cells/g)	Sample 2 (composite) (cells/g)
Aerobic BTEX and MTBE	-	-
Toluene/Benzene Dioxygenase (TOD)	<1.00E+04	<1.00E+04
Phenol Hydroxylase (PHE)	7.43E+06	2.31E+05
Toluene 2 Monooxygenase/Phenol Hydroxylase (RDEG)	2.86E+06	6.70E+04
Toluene Ring Hydroxylating Monooxygenases (RMO)	7.27E+04	<1.00E+04
Xylene/Toluene Monooxygenase (TOL)	<1.00E+04	<1.00E+04
Ethylbenzene/Isopropylbenzene Dioxygenase (EDO)	<1.00E+04	<1.00E+04
Biphenyl/Isopropylbenzene Dioxygenase (BPH4)	9.67E+04	<1.00E+04
Methylibium petroliphilum PM1 (PM1)	4.80E+08	6.59E+06
TBA Monooxygenase (TBA)	<1.00E+04	4.92E+04
Aerobic PAHs and Alkanes	-	-
Naphthalene Dioxygenase (NAH)	<1.00E+04	<1.00E+04
Phenanthrene Dioxygenase (PHN)	<1.00E+04	<1.00E+04
Alkane Monooxygenase (ALK)	<1.00E+04	<1.00E+04
Alkane Monooxygenase (ALMA)	<1.00E+04	<1.00E+04
Anaerobic BTEX	-	-
Benzoyl Coenzyme A Reductase (BCR)	8.20E+08	7.06E+07
Benzylsuccinate synthase (BSS)	<1.00E+04	<1.00E+04
Benzene Carboxylase (ABC)	<1.00E+04	<1.00E+04
Anaerobic PAHs and Alkanes	-	-
Naphthylmethylsuccinate Synthase (MNSSA)	<1.00E+04	<1.00E+04
Naphthalene Carboxylase (ANC)	<1.00E+04	<1.00E+04
Alklysuccinate Synthase (ASSA)	<1.00E+04	<1.00E+04
Other	-	-
Dehalococcoides spp. (DHC)	2.34E+04	<1.00E+03
Total Eubacteria (EBAC)	1.32E+09	9.19E+07
Sulfate Reducing Bacteria (APS)	<1.00E+04	<1.00E+04

4.0. Laboratory Microcosm Experiments

4.1. Methods used for Microcosm Experiments

4.1.1. Soil Sample Location Selection and Prescreening Soil Collection

Soils used in the microcosms were collected from SSFL Area IV on January 16, 2014. Soil sample locations were selected to provide moderate COI concentrations based on historical sampling. Soils were also selected to have minimal concentrations of metals to prevent potential toxicity to microbes. Once potential soil collection locations were identified, the locations were prescreened for total organic vapors using a calibrated photoionization detector. Background readings were recorded prior to the start of sampling, and additional readings were taken during sampling. Collection locations were also prescreened for residual radiation using a MicroR gamma detector and Dual Phosphor Alpha Scintillator (alpha/beta detector). Gamma, alpha, and beta measurements were collected approximately 0.5-1 inch above the ground surface of the sample area. Once locations were determined to be free of radiation, pre-screening soil samples were also collected to ensure that treatability study samples were not taken from soils with COI concentrations exceeding federal or state regulatory levels for hazardous wastes and to compare actual soil concentrations to target concentrations. Soil sample collection was conducted by Hazardous Waste Operations and Emergency Response (HAZWOPER)-certified field personnel per 29 CFR 1910.120. Soils initially selected had COI concentrations lower than target values, so other locations were selected for soil collection. Soil gas data and soil temperatures were collected from the site in the summer of 2014. Soil properties including total organic carbon (TOC), total nitrogen, pH, and moisture were also measured and recorded during the first and second microcosm sampling events.

A total of 68 kg of soil was collected from three sample locations: 5C_DG-516, 5C_DG-755, and PUBS1044 from the locations and depths specified in Table 4.1 and Figure 4.1. Soils were collected using stainless steel shovels and placed in Teflon-lined 5-gallon buckets for transport to Cal Poly.

Table 4.1: Soil sample locations for collecting soil for microcosm experiments

Soil Label	Sample location designation	Depth of collection (ft bgs)	Amount collected (kg)
A	5C_DG-516	4-5.5	52
B	PUBS104	1-4	8
C	5C_DG-755	1-4	8

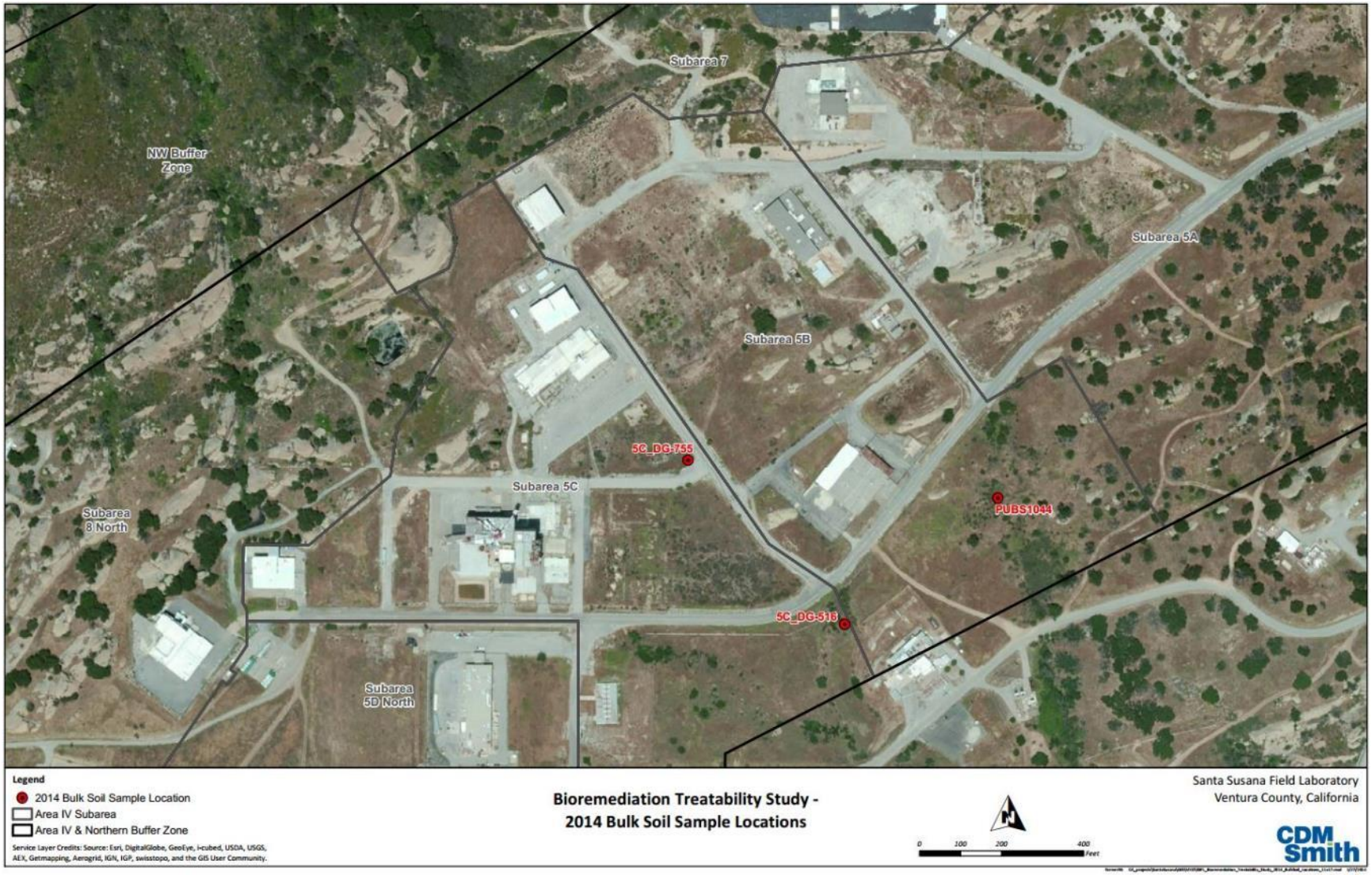

Figure 4.1: Bulk Soil Sample Collection Locations for Microcosms

Collected soils were sieved through a No. 4 sieve (4.76 mm). After sieving, soil was homogenized in 5-gallon increments in an acid-washed (10% weight by weight, w/w, HNO3 solution followed by triple-rinsing with DI water) 10-gallon UNS S30400 stainless steel drum. Soils were rolled in the drum in a well-ventilated area for five minutes and replaced in their respective Teflon-lined 5-gallon buckets. The drum was rinsed and air-dried between uses.

After sieving and homogenization, soil moisture was determined using ASTM Method D2216 (ASTM Standard D2216, "Standard Test Methods for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass" 2010). Soil samples were placed in a clean, dry, labeled container. A balance was used to determine the mass of the moist soil and container. This value was recorded. The moist soil was then placed in a drying oven at 105° overnight. Once dry, the soil was removed from the oven and reweighed using the same balance. This oven-dried mass was recorded and used to determine the water content of the sample.

4.1.2. Microcosm Preparation

Forty five soil microcosms were prepared in Fisher Scientific™ glass jars with Teflon™-lined lids. The jars and lids were acid washed in a 10% w/w nitric acid solution and triple rinsed in milliQ deionized water and air dried. To prepare each microcosm, 1.4 kg of homogenized soil was placed in each acid-washed glass jar.

Amendments were added to each microcosm as described in Table 4.2 and thoroughly mixed using a stainless steel spatula. Moisture content was adjusted to 15% (w/w) by adding deionized water. The amount of water to add was calculated based on the measured soil moisture content. After moisture and amendments were added to each microcosm, they were thoroughly mixed with a stainless steel trowel, sealed with a Teflon-lined lid, and shaken to evenly distribute soil. Five replicates of each type of microcosms were prepared to provide for the statistical analysis.

To provide sterile controls, five of the microcosms containing soil from collection location 5C_DG-516 (Soil A) and milliQ water (for 15% moisture) were transported to Sterigenics, a sterilization facility, located in Gilroy, California. Microcosms were dosed with 25 kilograys using Cobalt-60 irradiation to ensure adequate sterilization (Abo-El-Seoud et al. 2004).

Table 4.2: Microcosm amendments and experimental design (each set of microcosms was comprised of 5 replicates)

Microcosm ID	Description	Amendments	Abbreviation	Amount Amendment Added	Sterile?	Collection Location
A1	Fertilized	Nutrient solution	NUTRIENT	0.1g KH_2PO_4 0.015g $MgSO_4 \cdot 7H2O$ 0.02g $CaCl_2 \cdot 2H_2O$ 0.29g $NaNO_3$	No	5C_DG-516
A2	Surfactant	Soya lecithin	SURFACT	1.5% w/w	No	5C_DG-516
A3	Bulking agent	Rice hulls	RICEHULL	10% w/w	No	5C_DG-516
A4	Bioaugmented	Rice hulls, Nutrient solution, Malt extract, *P. chrysosporium*	BIOAUGM	10% w/w rice hulls 0.1g KH_2PO_4 0.015g $MgSO_4 \cdot 7H2O$ 0.02g $CaCl_2 \cdot 2H_2O$ 0.29g $NaNO_3$ 0.15g malt extract *P. chrysosporium*	No	5C_DG-516
A5	Combined Amendments	Rice hulls, Nutrient solution, Malt extract, *P. chrysosporium*, Soya lecithin	COMB	10% w/w rice hulls 0.1g KH_2PO_4 0.015g $MgSO_4 \cdot 7H2O$ 0.02g $CaCl_2 \cdot 2H_2O$ 0.29g $NaNO_3$ 0.15g malt extract *P. chrysosporium* 1.5% soya lecithin	No	5C_DG-516
A6	Unamended A	None	UNAMENDA	None	No	5C_DG-516
A7	Sterilized	Gamma irradiation	STERILE	25 kilograys of gamma irradiation	Yes	5C_DG-516
B6	Unamended B	None	UNAMENDB	None	No	PUBS1044
C6	Unamended C	None	UNAMENDC	None	No	5C_DG-755

4.1.3. Microcosm Incubation

Microcosms were incubated in a U-Line stainless steel cabinet lined with polyisocyanurate foam board insulation. Temperature was kept constant in this cabinet using a temperature-controlled water bath with tubing routed throughout the shelving. Microcosm temperature was measured and recorded weekly using a HDE high accuracy non-contact Fluke infrared IR thermometer gun with laser sight. Soil temperature was also measured and recorded directly with a standard thermometer in an extra microcosm that contained no amendments. This temperature reading confirmed that the infrared thermometer's measurements were representative of actual soil temperature. Incubation temperatures are reported below with results.

The microcosm experiment ran for a total of twelve months. Soil samples were collected for analysis of all COIs after 0, 4 and 8 months of incubation. The sterilized controls were only sampled after 8 months to avoid contaminating these microcosms with microorganisms during the experiment. Petroleum hydrocarbon concentrations (EFH) were measured after 12 months of incubation because there was an analytical problem with the 8-month EFH samples.

4.1.4. Soil Sample Collection and Analysis

Soil sampling was conducted using a stainless-steel trowel that was pre-washed with Alconox and triple-rinsed with ASTM Type II Water (reagent grade water defined by American Standards for Testing and Measurements that is used in the final rinse of surfaces of contaminated equipment) between microcosm types. All non-disposable sampling equipment used was decontaminated using a decontamination line. The line consisted of three buckets: one for scrubbing Alconox solution on the equipment with a stiff bristle brush (to remove particulate matter and surface films), one for rinsing off dirt and Alconox with ASTM Type II Water. At the end of sampling activities, all laboratory-derived waste was collected, labeled as such, and transported back to SSFL for proper disposal.

Samples were transported to EMAX and Lancaster laboratories for analysis using the analytical methods listed in Table 4.3.

4.1.5. Microcosm Data Analysis

Following sample collection and soil analysis, the resulting data were checked for quality control by CDM Smith personnel. Statistical analyses were performed using Minitab. Average, standard deviation, and standard error of contaminant concentrations (both summations of individual compounds within a contaminant type and individual compounds) were calculated.

Statistical analysis of data included a general linear model with the response variable being either chemical concentration, log (concentration), or the square root of concentration. The log (concentration) and square root of concentration were calculated in an attempt to normalize data

if fanning of residuals was observed. The general linear model used for this analysis analyzed the statistical significance of treatment's effect on contaminant concentration at the three different sampling events. Residual plots provided a helpful visual representation of data normality. The general linear model was used to compare three different sets of data:

- Effect of treatment on changes in contaminant concentrations in soil A
- Effect of gamma irradiation on changes in contaminant concentrations in soil A (using only beginning and end time points to include gamma irradiated samples were not analyzed at the sampling midpoint), and
- Effect of different soil type (A, B, or C) on changes in contaminant concentrations over time.

Table 4.3: Analytical methods used for soil sample analysis

Analyte	Analytical Methods for Soil	Laboratory (sampling date)
PCBs	EPA Method 8082A Gas Chromatograph/Electron Capture Detector	EMAX (0, 126 days) Lancaster (244 days)
Dioxins	EPA Method 1613B Gas Chromatograph/High Resolution Mass Spectroscopy	Lancaster (0, 126, 244 days)
PAHs	EPA Method 8270C/D SIM Gas Chromatograph/High Resolution Mass Spectroscopy	EMAX (0, 126 days) Lancaster (244 days)
TPH	EPA Method 8015B/C/D Gas Chromatograph/Flame Ionization Detector	EMAX (0, 126 days) Lancaster (244 days) EMAX (362 days)
Metals	EPA Method 6010C/6020A/7471B Inductively Coupled Plasma-Atomic Emission Spectrometry, Inductively Coupled Plasma-Mass Spectrometry Mercury in Solid or Semisolid Waste (Manual Cold-Vapor Technique)	EMAX (0, 126 days)
Mercury	Cold vapor atomic absorption spectroscopy EPA Method 7471B	EMAX (0, 126 days)
Percent Moisture	ASTM D2216 Standard Test Methods for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass	Lancaster (0, 126, 244 days)

Table 4.3 Continued…

Analyte	Analytical Methods for Soil	Laboratory (sampling date)
Nitrogen	ASTM D5373 Standard Test Methods for Determination of Carbon, Hydrogen and Nitrogen in Analysis Samples of Coal and Carbon in Analysis Samples of Coal and Coke	Lancaster (0, 126 days)
Organic Carbon	SM 5310B Total Organic Carbon	Lancaster (0, 126 days)

4.2. Results of Microcosm Experiments

This section of the report describes first the site conditions (Section 4.2.1) and then the results of the microcosm experiments (Section 4.2.2). Characterization of the soil collected for the microcosms is described in the beginning of Section 4.2.2.

4.2.1. Characterization of Site Conditions

Soil vapor composition: Soil gas data collected in June and July of 2014 indicate that average oxygen concentrations at 1-foot intervals in soil vapor ranged from 10.1% to 20.0%, and the lowest average concentration was detected at 20-21 feet below ground surface (bgs) (Table 4.4). This indicates that aerobic conditions prevail at the site. There could however be small anaerobic zones on soil particles. The maximum carbon dioxide concentration (6.3%) was detected at 20 ft bgs (Table 4.4). The high carbon dioxide concentrations are an indicator of extensive biological respiration – either of contaminants or natural organic material.

Table 4.4: Soil Gas Composition at the site (June 2014)

Depth Interval (ft bgs)	Carbon Dioxide (%)		Oxygen (%)	
	Average	Standard Deviation	Average	Standard Deviation
5-6	1.8	2.4	18.3	3.5
6-7	2.0	1.5	16.9	5.0
7-8	2.1	1.4	17.4	3.9
8-9	1.5	1.4	18.4	1.2
9-10	2.9	2.5	17.2	3.3
10-11	3.0	3.2	15.5	6.2
11-12	2.5	1.7	17.5	1.6
12-13	3.2	2.2	13.9	8.1
13-14	1.2	1.6	18.4	1.5
14-15	1.0	*	15.9	*
15-16	4.0	4.5	14.5	6.9
16-17	1.6	*	19.0	*
17-18	0.0	*	20.0	*
18-19	2.4	1.1	16.6	0.1
19-20	4.6	*	16.2	*
20-21	6.3	3.8	10.1	7.7
*only one measurement taken at this depth interval				

Soil temperature: Site soil temperatures were measured in May and June of 2014. Overall average site soil temperature was 30ºC with a standard deviation of 7ºC (Table 4.5). Soil temperature varied greatly with vegetative cover because of shading. Soil temperature decreased with increasing depth (Table 4.5).

Table 4.5: Soil Temperature Data (Summer 2014)

Location ID	Depth (ft)	Time	Temp (°C)	Date
STS-18_CB_A	0	8:15	23	6/2/2014
STS-06_PG_C	0	8:28	23	6/4/2014
STS-01_BE_D	0	8:47	26	6/2/2014
STS-04_MF_B	0	9:33	26	6/3/2014
STS-17_NM_C	0	9:45	26	6/4/2014
STS-02_LS_B	0	10:22	30	6/3/2014
STS-04_MF_D	0	10:55	29	6/2/2014
STS-01_BE_C	0	11:35	31	6/4/2014
STS-23_YS_C	0	12:50	34	6/3/2014
STS-06_PG_D	0	13:40	37	6/2/2014
Average			**29**	
Standard Deviation			**5**	
STS_35_NG_C	0.1	7:30	22	5/28/2014
STS_08_SM_CC	0.1	8:10	22	5/29/2014
STS-01_BE_A	0.1	8:30	23	5/29/2014
STS_17_NM_BB	0.1	8:45	24	5/28/2014
STS-23_YS_D	0.1	8:50	25	5/30/2014
STS-18_CB_D	0.1	9:35	27	5/30/2014
STS_08_SM_D	0.1	9:36	35	6/2/2014
STS_08_SM_BB	0.1	9:50	28	5/28/2014
STS_35_NG_A	0.1	10:30	26	5/27/2014
STS-23_YS_A	0.1	10:50	37	5/29/2014
STS-02_LS_D	0.1	11:00	30	5/30/2014
STS-17_NM_D	0.1	12:15	49	5/30/2014
STS-02_LS_C	0.1	12:40	35	5/28/2014
STS_35_NG_B	0.1	13:30	37	5/27/2014
STS-06_PG_B	0.1	14:15	44	5/29/2014
STS_35_MG_D	0.1	14:25	36	5/28/2014
Average			**31**	
Standard Deviation			**8**	
STS-18_CB_A	1.5	8:15	24	6/3/2014
STS-01_BE_D	1.5	8:47	27	6/2/2014
STS-23_YS_D	1.5	8:50	30	5/29/2014
STS-23_YS_A	1.5	10:50	29	5/29/2014
STS-04_MF_D	1.5	10:55	25	6/2/2014
Average			**27**	
Standard Deviation			**3**	
Overall Average			**30**	
Overall Standard Deviation			**7**	

4.2.2. Microcosm Soil Characteristics and Conditions

Soil pH, TOC, total nitrogen, and moisture content were all measured during February and June microcosm sampling events in 2014.

Microcosm soil pH: The pH of the microcosm soils was within the range of 5.8-7.4 (Table 4.6). Two microcosm sets (A2 and A5) had an initial pH outside of the EPA's specified acceptable pH region for optimal bioremediation (6-8). These two microcosm types included soya lecithin as an amendment which may have caused the reduced pH. This indicates that soy lecithin could have an adverse effect on initial degradation unless pH is adjusted. Over time, though, the pH in soy lecithin-amended microcosms increased to within the acceptable range (Table 4.2).

Table 4.6: Microcosm Soil pH

	Average		**Standard Deviation**		**Standard Error**	
Microcosm Type	0 days	126 days	0 days	126 days	0 days	126 days
Nutrient	6.53	6.28	0.04	0.06	0.02	0.03
soya lecithin	5.87	6.31	0.03	0.19	0.01	0.08
rice hulls	6.60	6.24	0.07	0.17	0.03	0.08
nutrients+ rice hulls+ *P. chrysosporium*	6.44	6.35	0.06	0.18	0.03	0.08
nutrients+ soya lecithin+ rice hulls+ *P. chrysosporium*	6.03	6.18	0.03	0.09	0.01	0.04
unamended Soil A	6.64	6.30	0.03	0.02	0.01	0.01
unamended Soil B	6.84	6.68	0.03	0.05	0.01	0.02
unamended Soil C	7.35	7.33	0.05	0.05	0.02	0.02
gamma-irradiated unamended Soil A	6.676	N/A	0.038471	N/A	0.017205	N/A

Microcosm soil total organic carbon: Soil total organic carbon (TOC) concentrations in microcosm soils varied for the different microcosm sets due partly to the effects of the amendments (Appendix H). The initial TOC in Soil B was much greater than that in Soil A and Soil C. TOC decreased slightly in almost all of the microcosms suggesting some biodegradation, but there was a large amount of variability, particularly during the initial sampling event and for both sampling events in the unamended Soil B.

Microcosm nitrogen: Total nitrogen concentrations in the microcosm soils varied significantly among microcosm sets (Appendix H). During incubation, nitrogen concentrations either remained unchanged or slightly increased. This indicates that there were sufficient nitrogen nutrients in the soil, and contaminant degradation was not nitrogen-limited. Phosphorus concentrations were not measured, so it is not known if phosphate was limiting biodegradation.

Microcosm soil moisture: Target experimental soil moisture in the microcosms was 15% based on previous research (Rastegarzadeh, Nelson, and Ririe 2006). As data from previous sampling events was received and analyzed, soil moisture was adjusted in an attempt to meet the target 15%. As a result, soil moisture was maintained between a minimum of 9% and a maximum of 17% throughout the experiment (Appendix H). Moisture content can be a limiting factor in biodegradation; however, lower moisture content is likely more representative of actual site conditions due to the low rainfall at the site.

Microcosm Incubation Temperature: Microcosm soil temperatures throughout the study averaged 27.4ºC with a standard deviation of 3.1ºC (Figure 4.2). This is slightly lower than the average site temperature observed in June and July, but presumably much higher than soil temperatures in the winter. This suggests that any biodegradation rates observed in microcosm data could be slightly elevated estimations of what could happen if a bioremediation technology were applied at the site year-round.

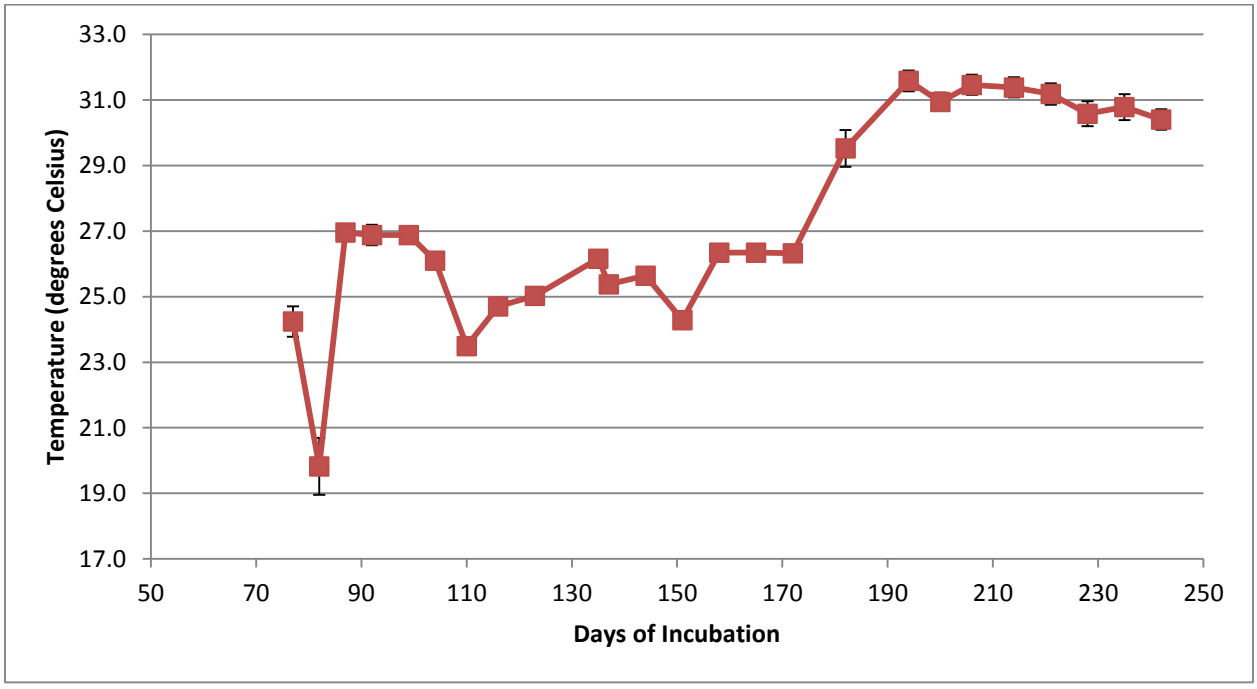

Figure 4.2: Microcosm soil incubation temperatures

Initial soil COI concentrations in microcosms: Initial soil COI concentrations in the microcosm soils were much lower than the target concentrations. Total EFH concentrations ranged from 100 to 230 mg/kg, about 20 to 40 times higher than the current cleanup goal of 5.7 mg/kg of EFH (C15-C20). Initial PAH concentrations ranged from 87 to 45,139 µg/kg). PCB concentrations ranged from 37 to 328 µg/kg as Aroclor 1260, and consisted mostly of heavily chlorinated congeners. Initial dioxin concentrations ranged from 0.026 – 0.116 mg/kg.

4.2.3. Microcosm COI Biodegradation Results

The total concentration of each contaminant type (petroleum hydrocarbons, PAHs, PCBs, and dioxins) were calculated and the averages and standard errors were plotted as a function of time to examine overall trends in chemical concentrations and overall concentrations of each COI are reported in this section of the report. More detailed results for each individual chemical (for example each PCB congener and each PAH compound) are also provided in graphical form in Appendices D – H, and in tabular form in Appendix I. The abbreviations for the conditions in each set of microcosms are defined in Table 4.7.

Table 4.7 Microcosm descriptions and treatments

Microcosm ID	Microcosm Description
NUTR	Soil A + nutrients (NPK)
SOLE	Soil A + soya lecithin (surfactant
RICE	Soil A + rice hulls (bulking)
AUGM	Soil A + nutrients + rice hulls + *P. chrysosporium*
COMB	Soil A + nutrients + soya lecithin + rice hulls+ *P. chrysosporium*
UNAA	Soil A unamended
UNAB	Soil B unamended
UNAC	Soil C unamended
STER	Soil A sterilized by gamma-irradiation (unamended)

Petroleum hydrocarbons: Initial EFH concentrations were elevated in microcosms containing soy lecithin because the soy lecithin eluted at the same time as petroleum hydrocarbons during gas chromatography (Figure 4.3). These inflated EFH concentrations decreased at the second sampling event, suggesting that the soy lecithin biodegraded during the first 4 months of microcosm incubation. Nonetheless, it was not possible to ascertain EFH biodegradation in the microcosms with soy lecithin amendment because of this interference. Thus EFH concentrations were not measured for microcosms with soy lecithin at 8 months.

EFH concentrations at 0 and 4 months were measured by EMAX Laboratory, and EFH was measured at 8 months by Lancaster Laboratory. EFH concentrations for all microcosms appeared to be about 5 times greater at the 8-month sampling (measured by Lancaster) compared to initial or 4-month samples analyzed by EMAX. There appears to be a difference in the data analysis methods between these two laboratories that caused this anomaly. For example, the two laboratories may have used different methods of integration or established a different baseline for integrating the chromatograms. Because of this apparent anomaly, microcosm soils were re-sampled at 12 months and these samples were analyzed for EFH only by EMAX, so that EFH concentrations could be compared based on one laboratory's results.

For the unamended soils, no significant overall decrease in total EFH concentration was observed. For Soil A, EFH appeared to decrease over the first 126 days and then increased again at 362 days, suggesting that the observed decrease was likely due to variability of EFH concentrations in the soil. Amendment of Soil A with nitrate and phosphate fertilizers appeared to result in a significant decrease in EFH concentration (Figure 4.3). This effect was less pronounced for the microcosms receiving a combination of nutrients, rice hulls and surfactant. Other amendments, such as bioaugmentation with fungus, resulted in lower final EFH concentrations, but these effects were not statistically significant at the 95% confidence level.

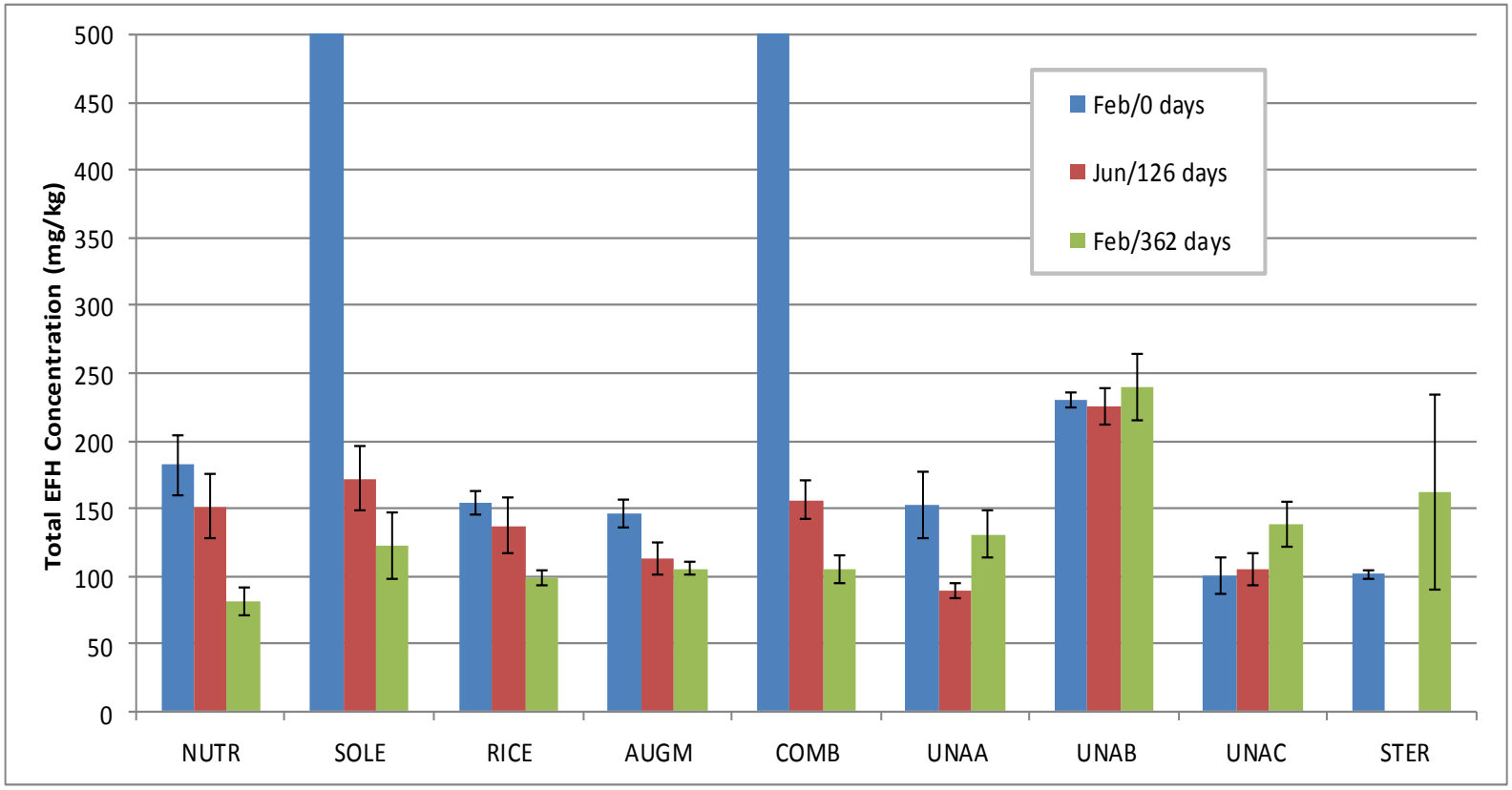

Figure 4.3: Total EFH concentration during microcosm incubation. Error bars indicate standard error of the mean. EFH concentrations for microcosms with surfactant added (SOLE) are truncated because the soy lecithin created an anomalous EFH concentration. Column labels defined in Table 4.7 above.

PAHs: Total PAH concentrations were calculated by summing all of the individual PAH concentrations, as given in Appendix F. Initial PAH concentrations were much higher in unamended Soil B than either Soil A or Soil C (Figure 4.3). PAH concentrations decreased slightly in Soil B microcosms (Figure 4.4). However, observed decreases in PAH concentrations were not statistically significant with a 95% confidence level (p-value of 0.296). PAH concentrations for Soils A and C were plotted separately (Figure 4.5) to avoid being overshadowed by the high PAH concentrations of Soil B. For Soil C, total PAH concentrations appeared to decrease dramatically, but the high variability of PAH concentrations (as indicated by the large error bars in Figure 4.5) resulted in no statistical significance to this decrease. For Soil A, total PAH concentration appeared to actually increase in several of the amended microcosms. This is undoubtedly due to the high variability of PAH concentrations measured.

The PAH contamination in these soils is largely comprised of compounds with 4-6 aromatic rings (Appendix F), and these are typically the most recalcitrant PAHs (Llado et al. 2013). Biodegradation of lighter PAHs may have already occurred at the site. Also, once PAHs adsorb onto soils, their biodegradation becomes difficult as their bioavailability is reduced. Residual contamination may be tightly adsorbed onto the soil matrix. However, surfactant addition to two sets of microcosms (SOLE and COMB) did not enhance PAH biodegradation (Figure 4.4).

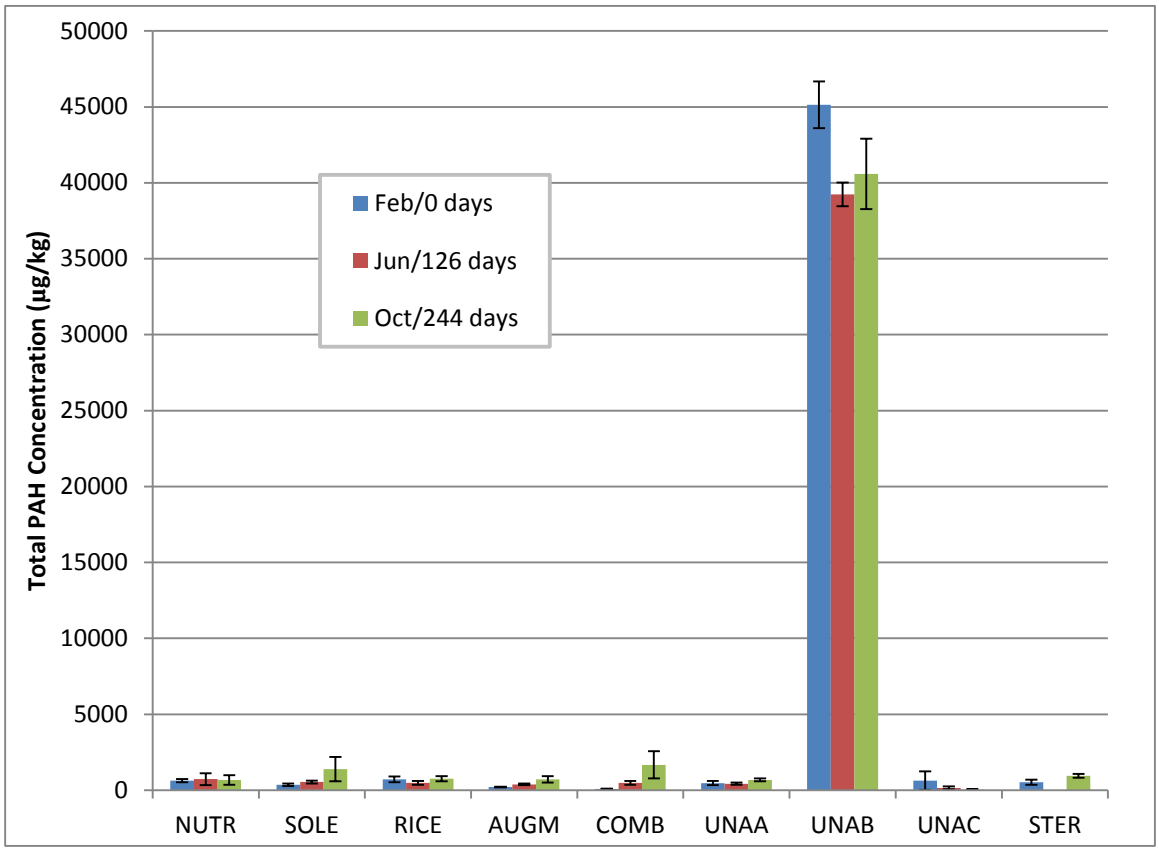

Figure 4.4: Total PAH concentration during microcosm incubation (all 3 soils). Error bars indicate standard error of the mean.
Column labels defined in Table 4.7 above.

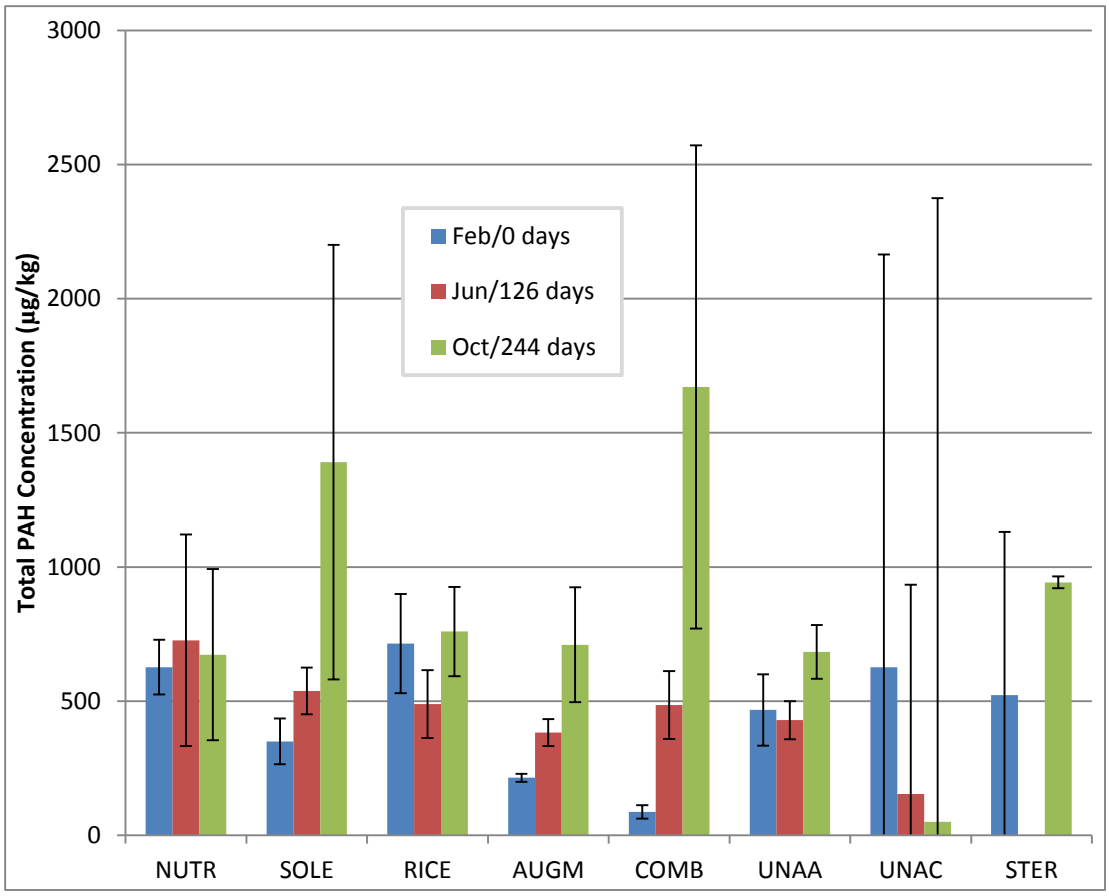

Figure 4.5: Total PAH concentrations during microcosm incubation (Soils A and C). Column labels defined in Table 4.7 above. Error bars indicate standard error of the mean.

PCBs: Large decreases in Aroclor 1260 PCB concentrations were observed in all but one of the microcosms (Figure 4.6). However, a similar decrease in PCB concentration was observed for the sterilized control. In fact, statistical comparison of treatments indicated that no treatment resulted in greater reduction in concentration than another at the 95% confidence level. It is possible that PCBs in the microcosms adsorbed to the glass surfaces of the microcosms. The lack of significant PCB biodegradation observed may be because the PCB congeners detected at the site are highly chlorinated (54-60% by weight). Lesser chlorinated PCBs with just 1-2 chlorines that have been shown to degrade under aerobic conditions are only about 30% chlorine by weight, and it is likely that these compounds if present historically at the site would have already been biodegraded, leaving the more heavily chlorinated, recalcitrant compounds in the soil. The predominantly aerobic conditions at the site and in the microcosms make bacterially-mediated reductive dechlorination unlikely. Fungi such as *P. chrysosporium* and *Sphingomonas wittichii* have been shown to biodegrade PCBs under aerobic conditions, but in these experiments bioaugmentation with *P. chrysosporium* did not result in

significantly more PCB degradation (Figure 4.6). Another possible limitation of PCB biodegradation is sequestration in the soil which limits bioavailability. However, even addition of soy lecithin as a surfactant to release PCBs from the soil structure did not facilitate significant PCB degradation.

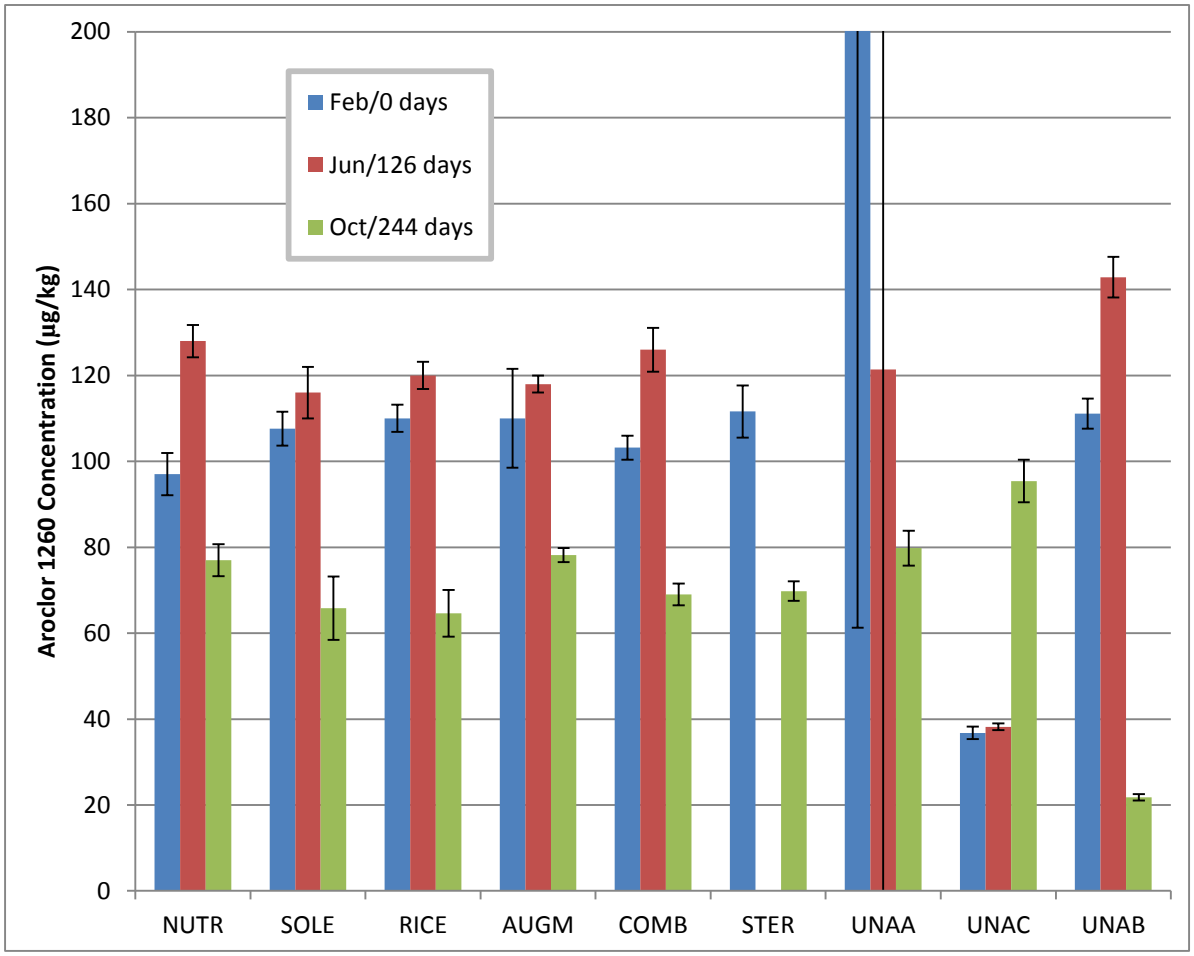

Figure 4.6: PCB (Aroclor 1260) concentration during microcosm incubation.
Column labels defined in Table 4.7 above.
Error bars indicate standard error of the mean.

Chlorinated Dioxins and TCDD TEQ: For the most part, total chlorinated dioxin concentrations did not decrease significantly for any of the soils or treatments over 8 months of incubation in the soil microcosms (Figure 4.7). Only the soils amended with the fungi *P. chrysosporium* (AUGM) showed any appreciable decrease in chlorinated dioxin concentration in this experiment. This suggests that bioaugmentation with this fungi may accelerate biodegradation of chlorinated dioxins at the site. While this is encouraging, even this decrease in dioxin concentration was not statistically significant at the 95% confidence level because of variability in the measured soil dioxin concentrations. Also, for microcosms amended with *P. chrysosporium* and soy lecithin (COMB), no appreciable decrease in dioxin concentration was observed (Figure 4.7). It is not clear why soy lecithin would interfere with biodegradation, unless its biodegradation consumed some nutrient needed for biodegradation. The sterile control held a constant dioxin concentration (Figure 4.7).

The predominant dioxin contaminant at the site is OCDD, which is the most heavily chlorinated dioxin congener. These highly chlorinated dioxins require anaerobic conditions to be bacterially dechlorinated, but site and experimental conditions were aerobic. Biodegradation under aerobic conditions may be possible with fungi such as *P. chrysosporium*, and indeed bioagumentation with this fungi appears to have aided dioxin biodegradtion, but again this observation was not statistically significant.

The dioxin source at the site could be from natural fires, or from anthropogenic sources. According to a paper citing congener profiles for anthropogenic sources of chlorinated DD/DFs, OCDD is the primary congener emitted from several industrial sources: municipal solid waste incineration with dry scrubbers and fabric filters for dioxin controls, industrial oil-fired boilers, industrial wood-fired boilers, unleaded gasoline combustion, diesel fuel combustion, and from sewage sludge incineration (Cleverly et al. 1997). Burning of hazardous waste results in minor OCDD and OCDF stack emissions. However, savanna woodland and arid grassland fires also produce DD/DFs dominated by OCDD (MacDougall, Rillig, and Klironomos 2011). Savanna woodlands seem to resemble site conditions (a grassland ecosystem with trees spaced so that the canopy does not close, seasonal water availability, and in the transitional zone between forest and desert or grassland) suggesting that emissions from a wildfire at SSFL might have contributed to the OCDDs as well.

TCDD TEQ, an important measure of dioxin congeners' toxicity, did not appear to decrease for any of the treatments (Figure 4.8).

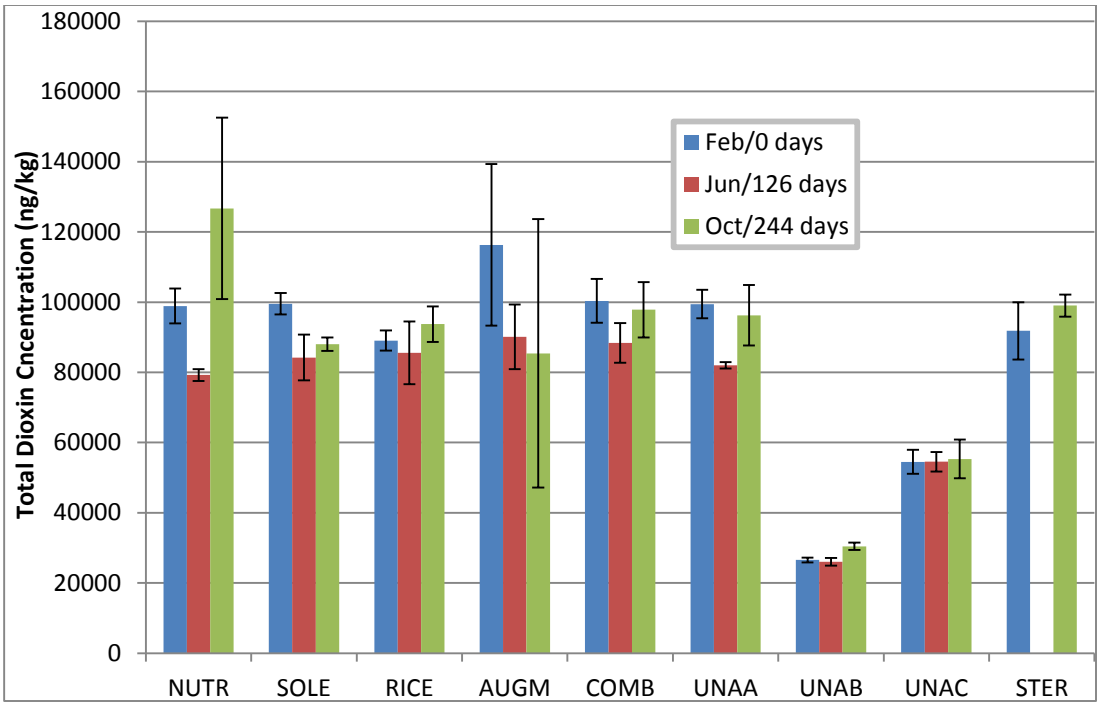

Figure 4.7: Total chlorinated dioxin concentrations during microcosm incubation. Error bars indicate standard error of the mean.

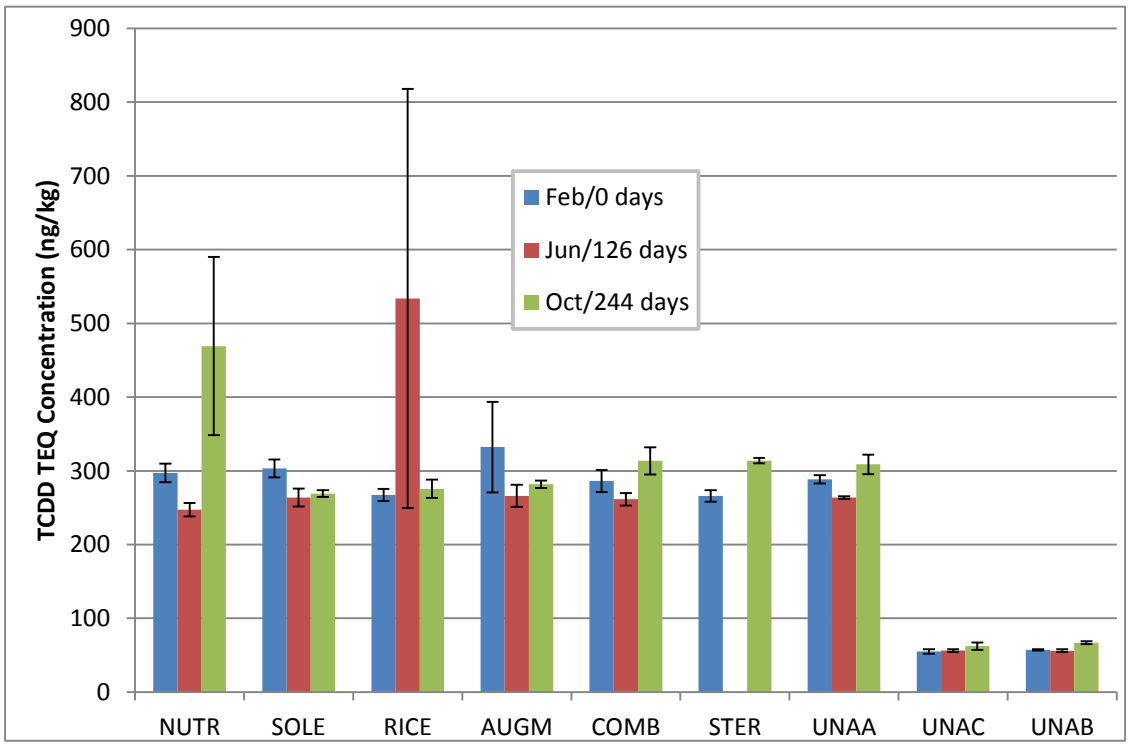

Figure 4.8: TCDD TEQ concentrations during microcosm incubation. Column labels defined in Table 4.7 above. Error bars indicate standard error of the mean.

4.2.4. Influence of Natural Organic Material on EFH analysis

Because of the observed discrepancy in results for EFH analyses by two commercial laboratories, the analysis of the petroleum hydrocarbons was explored using gas chromatography with mass spectroscopy in the Cal Poly laboratory. Soil from an unamended microcosm with Soil A was extracted into hexane and analyzed using an Agilent 5890 gas chromatograph with a mass spectrometer detector. The resulting chromatogram for this sample is shown in Figure 4.8. All of the larger peaks (Peaks 1-5 in Figure 4.9) gave mass spectra suggestive of organic acids, such as hexadecanoic acid (palmitic acid), which is a common fatty acid found in many animal, plant and microbial products. This compound was identified with a 77% probability. Stigmastan 3-5 diene was identified with an 85% probability, and this is a compound found in cooking oils – particularly after heating. Much smaller peaks identified in the background parts of the chromatogram (labelled "Flat 1-3") appear to be from high molecular weight hydrocarbons with branching and cyclic rings. These high MW hydrocarbons are exactly what you would expect for highly weathered petroleum compounds – since branching and ring structures limit biodegradability. This preliminary analysis was not quantitative, but it is likely from this analysis that a large portion of what is being reported as EFH is actually natural organic material (NOM).

The results of this preliminary analysis indicated the presence of significant amounts of natural organic material (NOM) in the soil which is likely to be counted as total petroleum hydrocarbon (TPH) and/or extractable fuel hydrocarbons (EFH) in standard tests for petroleum hydrocarbons. This interference could potentially result in reported TPH/EFH soil concentrations much higher than that attributable to hydrocarbons truly from petroleum-based origins. An in-depth study is currently underway at Cal Poly to quantify the NOM relative to EFH using silica gel fractionation methods.

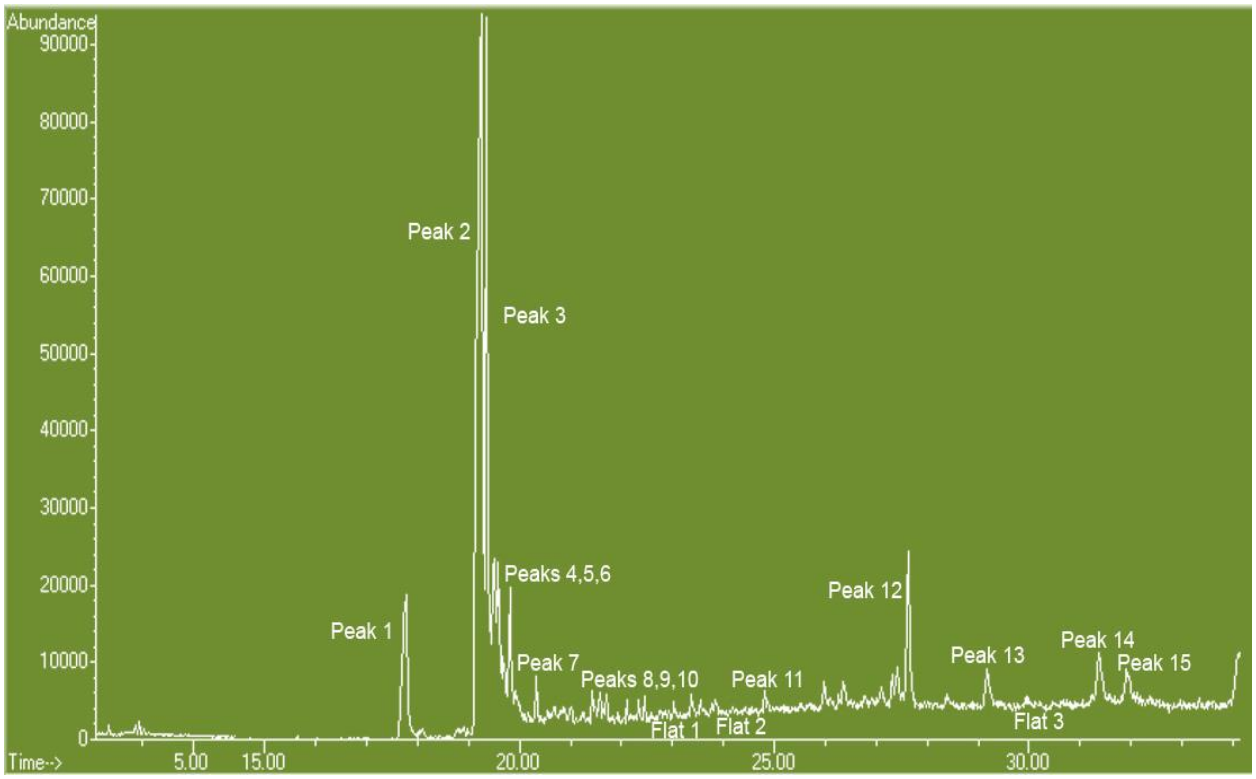

Figure 4.9: GC/MS Chromatogram of Soil A extracted into hexane.

5.0. Conclusions

Field assays of the microbial community in Area IV soils using both traditional culturing techniques and a variety of DNA-based analyses suggest that active bacteria and fungi are growing in soils at the site which are capable of biodegrading the COIs. Many species of bacteria and fungi were isolated through laboratory culturing by growing microorganisms on agar plates containing the COIs. These microorganisms were positively identified using sequencing techniques and found to include several genera of bacteria known to biodegrade hydrocarbons, including *Pseudomonas, Arthrobacter, Streptomyces, Micromonospora, and Variovorax.* Quantitative PCR (qPCR) analyses also indicated the presence of aerobic hydrocarbon degraders. Also, several strains of fungi, such as the white-rot fungus *Phanerochaete chrysosporium,* were cultured from the site soils. Strains of this fungi have been reported to be capable of PCB and chlorinated dioxin biodegradation under aerobic conditions (Pointing, 2001).

Bacterial biodegradation of highly chlorinated compounds, such as chlorinated dioxins and PCBs, is widely believed to occur only under anaerobic conditions (Bunge et al., 2003; Fennell et al. 2004; Krumins et al. 2009). Such anaerobic conditions facilitate the reductive dechlorination of these compounds by bacteria such as *Dehalococcoides* (Fennell, 2004). However, soil vapor analyses of Area IV soils indicate that these soils are highly aerobic, which favors petroleum hydrocarbon biodegradation, but not reductive dechlorination of dioxins and PCBs. Indeed, the qPCR analysis showed only a very small population of *Dehalococcoides* bacteria in one of the soil samples. It may be possible that there are anaerobic microenvironments in the soil which could harbor such microorganisms, but given the very small population of *Dehalococcoides* found with the qPCR analyses, this is likely an insignificant mechanism at the site.

Unlike bacteria, fungi do not require anaerobic conditions to biodegrade highly chlorinated dioxins and PCBs (Bento et al. 2005). This is somewhat encouraging because five of the fungal species isolated from Area IV soils have been shown to biodegrade chlorinated dioxins and PCBs. Bioaugmentation with *Phanerochaete chrysosporium* was tested in the microcosm experiments summarized below.

Terminal restriction fragment length polymorphism (TRFLP) assays did not show any significant correlations between microbial populations and COI concentrations. Similarly, sequencing of the soil biome of the site using metagenomics did not identify any strong correlations between specific bacterial or fungal genera and the COI concentrations. These results suggest that the populations described above may not be in large enough numbers to make a significant impact on TRFLP or metagenomics analyses, and possibly the overall site soil microbial community.

In the microcosm experiment, slight decreases in PAH, PCB, and dioxin soil concentrations were observed over 8 months of incubation, but these decreases were mostly not statistically significant at the 95% confidence level. For petroleum compounds, the EFH concentrations in unamended microcosms did not decrease significantly over the course of 12 months of incubation for all three soils tested. Addition of mitrate and phosphate fertilizer appeared to improve EFH biodegradation, suggesting that petroleum-degrading microorganisms may be limited by nutrient availability. However, fertilizer addition did not significantly enhance the biodegradation of the other COIs. Similarly, addition of rice hulls as a bulking material improved EFH biodegradation slightly, but did not enhance observed biodegradation rates of the other COIs. Since the purpose of bulking materials is to improve the aeration of soils, the limited effect of rice hulls suggests that the soils are well aerated and microorganisms are not likely to be limited by oxygen availability. This conclusion is also supported by soil vapor analyses which found oxygen levels from 10 to 20% at depths to 20 feet.

Bioaugmentation with *Phanerochaete chrysosporium* and biostimulation with soy lecithin as a surfactant appeared to improve dioxin degradation, although not at the 95% confidence level. Bioaugmentation did not appear to enhance the biodegradation of any of the other COIs.

In the case of petroleum hydrocarbons, natural organic material (NOM) appears to interfere with the EFH analysis. Preliminary mass-spectrometer analysis of these soils in the Cal Poly laboratory indicates that a significant amount of organic acids, such as hexadecanoic acid, may be contributing to erroneously high values of EFH because these compounds elute during the same time range as petroleum hydrocarbons. A detailed study of the effect of NOM on EFH analyses of site soils is underway at Cal Poly. Silica gel adsorption columns are being tested for their ability to remove the polar organic compounds from the soil extract solutions before GC analysis.

The low biodegradation rates observed in the microcosm experiment are likely due to the extensive weathering of the COIs due to 20-50 years of on-going natural attenuation processes at the site. One of the effects of weathering by these processes is the biodegradation of the most easily biodegraded compounds, leaving the more recalcitrant compounds (either original compounds or degradation products) in the soil over time. Because petroleum hydrocarbons were primarily longer-chain hydrocarbons in the C21 to C40 equivalent carbon range, it is likely that lighter hydrocarbons had been preferentially degraded, leaving the more recalcitrant longer-chain hydrocarbons in the soil. Similarly, the large PAHs (4-6 rings) may be somewhat recalcitrant and will take a long time to biodegrade. The limited PCB biodegradation was also not surprising because the PCBs detected at the site are heavily chlorinated, and bacterial biodegradation of these highly chlorinated compounds is reported to occur only under anaerobic conditions which were not observed in the field or in microcosms. Also, the most prevalent chemical form of the

chlorinated dioxin present in the soils was octachlorodibenzodioxin (OCDD), which is the most chlorinated form of dioxin. Like PCBs, this compound requires anaerobic conditions for reductive dechlorination, and such conditions are not present at the site as indicated by soil vapor analyses performed at the site. However, fungi could potentially biodegrade PCBs and chlorinated dioxins under aerobic conditions. Indeed, total chlorinated dioxin concentrations decreased in the microcosms amended with the fungi *Phanerochaete chrysosporium*, but this additional decrease was not statistically significant at the 95% confidence level.

Another effect of weathering is the sequestration of contaminants into the pore structure of the soil which can lower the bioavailability of contaminants to the microorganisms responsible for biodegradation. In some cases researchers have found that adding surfactants to soil can release contaminants from the soil structure and improve bioavailability. In this study addition of the soy lecithin as a natural surfactant improved biodegradation slightly. However, this effect was not statistically significant at the 95% confidence level.

In summary, while the field testing indicated the presence of bacterial and fungal species known to biodegrade the COIs, the laboratory microcosm experiments indicated that the biodegradation rates are low even with biostimulation and bioaugmentation. Nonetheless, the total time frame of the microcosm experiments was only 8-12 months, and more biodegradation could be expected over a longer time period. Future pilot tests could be conducted in the field to test biostimulation methods over a longer time frame and under conditions more closely matching those in the field. Given the slight improvement in biodegradation observed with fertilizing, bioaugmentation and addition of surfactant, these active bioremediation methods should be considered and may be worthy of field testing. In the end, bioremediation may be most suitable for locations at the site where long-term biodegradation is acceptable, such as in areas with low COI concentrations or areas with limited public exposure, and where the length of time required for reaching cleanup levels would not be an issue.

For petroleum hydrocarbons, it is possible that the true concentrations of petroleum compounds in the soils are lower than previously thought because of NOM interference, and knowledge of this interference could reduce the amount of soil that needs to be hauled off-site by providing more accurate and meaningful estimates of EFH concentrations.

6.0. References

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7.0. Appendices

Appendix A: Abbreviations

2,3,7,8-TeCDD	2,3,7,8-Tetrachlorodiebnzo-p-dioxin
AI	Atomic International
AOC	Administrative Order of Consent
ASTDR	Agency for Toxic Substances and Disease Registry
bgs	Beneath ground surface
BLAST	Basic Local Alignment Search Tool
BTEX	Benzene, toluene, ethylbenzene, xylenes
CBP	Chlorinated Biphenyl
COI	Contaminant of Interest
DBZ	Dibenzofuran
DD	Dibenzodioxin
DD/DF	Dibenzodioxins/dibenzofurans
DF	Dibenzofuran
DOD	Department of Defense
DOE	Department of Energy
DTSC	Department of Toxic Substances
EDTA	Ethylenediaminetetraacetic acid
EFH	Extractable Fuel Hydrocarbons
EPA	Enviromental Protection Agency
ETEC	Energy Technology Engineering Center
F	Field
HpCDD	Heptachlorodibenzo-p-dioxin
g	acceleration of gravity
kg	kilogram
Kow	Octanol Water Partition Coefficient
L	Lab
LiP	Lignin Peroxidase
MCDF	Monochlorinated Dibenzofurans
min	minute
mg	milligram
mm	millimeters
MDS	multidimensional scaling
MnP	Manganese-dependent Peroxidase
MTBE	Methyl Tertiary Butyl Ether
NAA	North American Aviation
NASA	National Aeronautics and Space Administration
NCBI	National Center for Biotechnology Information
ng	Nanogram
NPK	Nitrogen-phosphorus-potasium
NS	Not Stated
NSD	Not Significantly Different
OCDD	Octachlorodibenzodioxin

PAH	Polyaromatic Hydrocarbon
PCB	Polychlorinated Biphenyl
PCDD	Polychlorinated Diobenzodioxin
PCDFs	Polychlorinated Dibenzofurans
pg	picograms
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
RMHF	Radioactive Materials Handling Facility
s	second
SDS	sodium dodecyl sulfate
SSFL	Santa Susana Field Laboratory
STIG	Soil Treatability Investigation Group
TBA	Tetra Butyl Alcohol TBA
TCDD	Tetrachloro Dibenzodioxin
TeCDD	Tetrachloro Dibenzodioxin
TEF	Toxic Equivalency Factor
TEQ	Toxic Equivalents
TPH	Total Petroleum Hydrocarbon
µg	microgram
µL	microliter
USAF	United States Air Force
EPA	United States Environmental Protection Agency
UV	Ultra Violet
V	Volts
w/w	Weight over weight

Appendix B: Additional MDS Scatter Plots from TRFLP Analysis

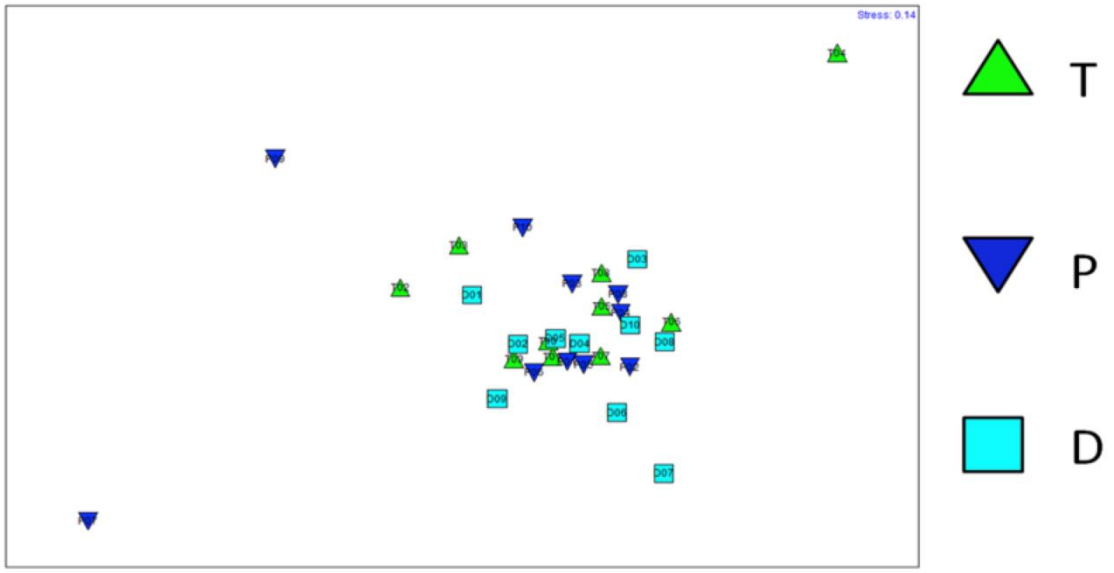

Figure B.1: MDS from bacterial fragments using COI series as a factor

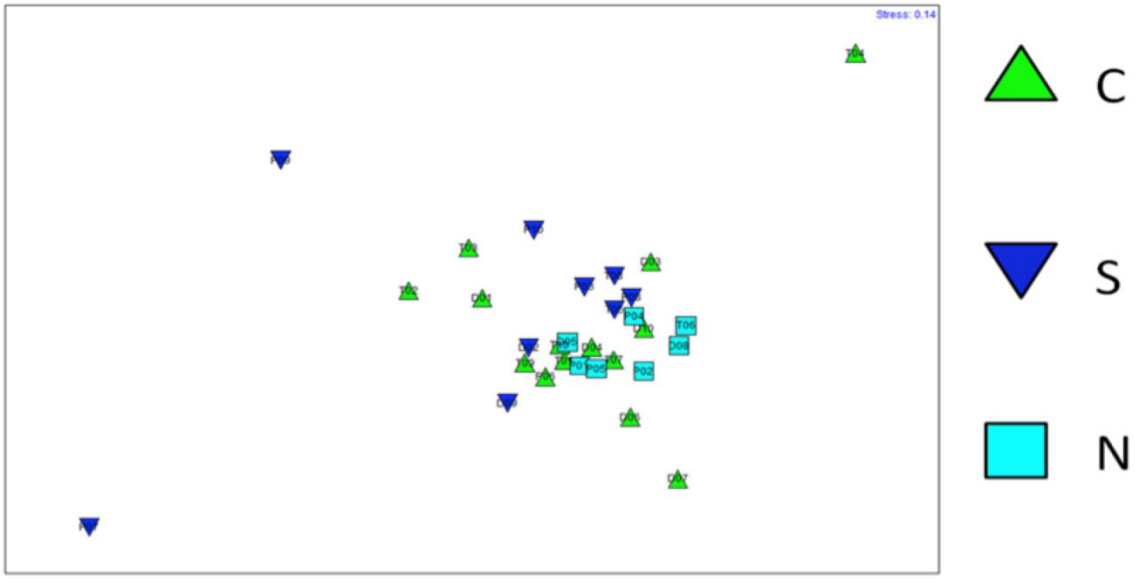

Figure B.2: MDS from bacterial fragments with added factor for location based on the map in Figure 3.1. Sites were labeled them generally south, central, and north.

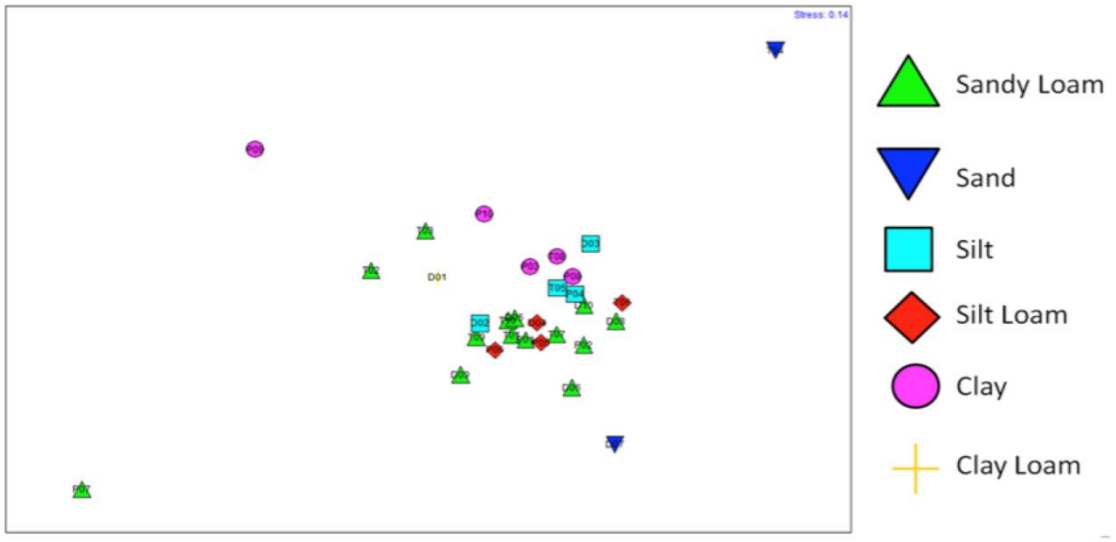

Figure B.3: MDS from bacterial fragments with soil types

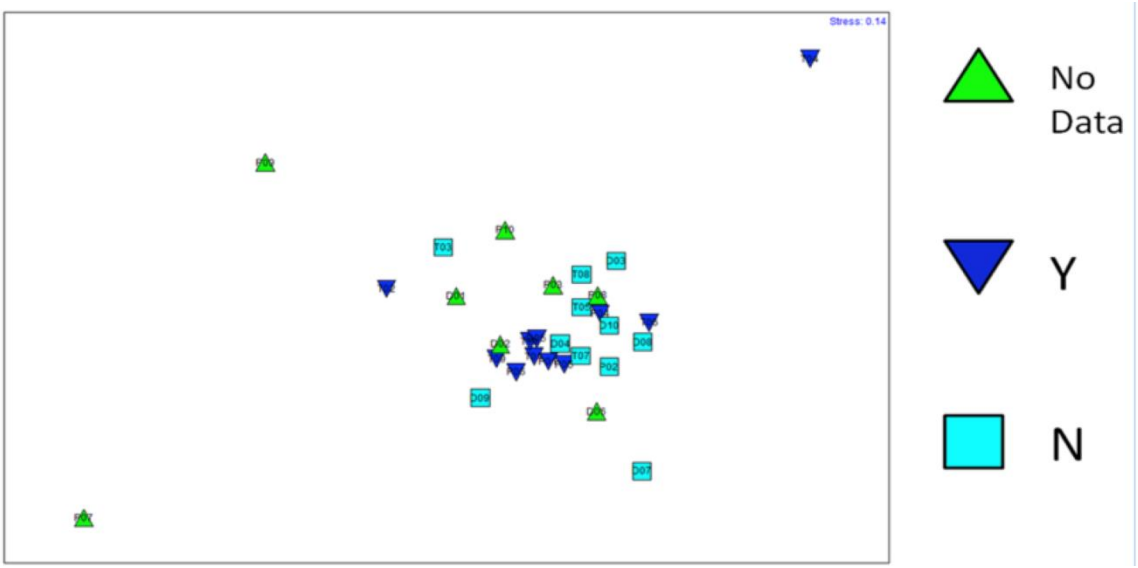

Figure B.4: MDS from bacterial fragments using presence/absence of TPH based on a threshold of 300 ppm.

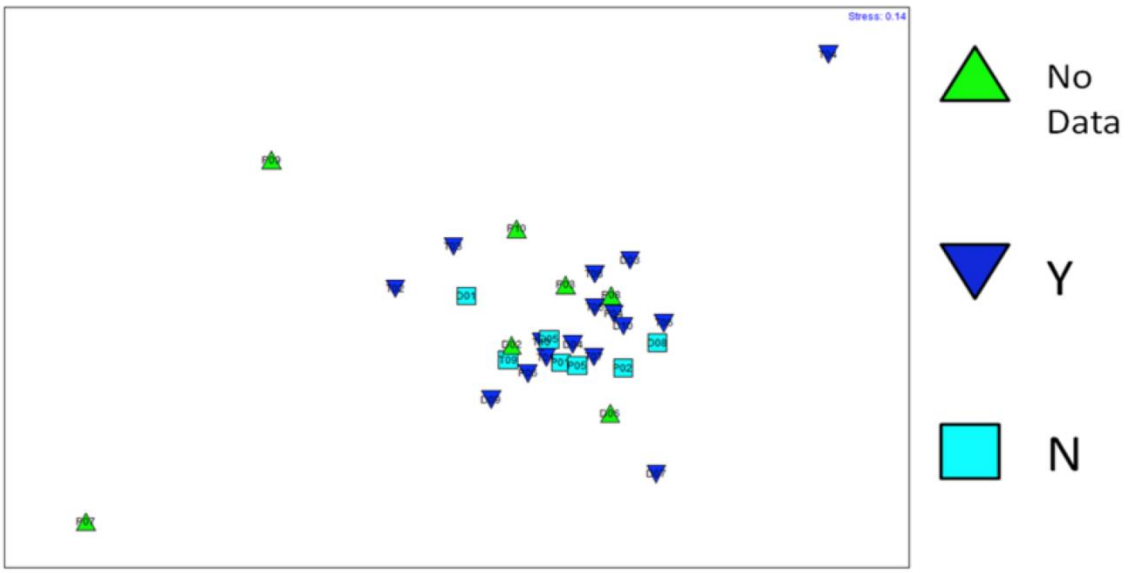

Figure B.5: MDS from bacterial fragments using presence/absence of PAHs based on a threshold of 2.5 ppm.

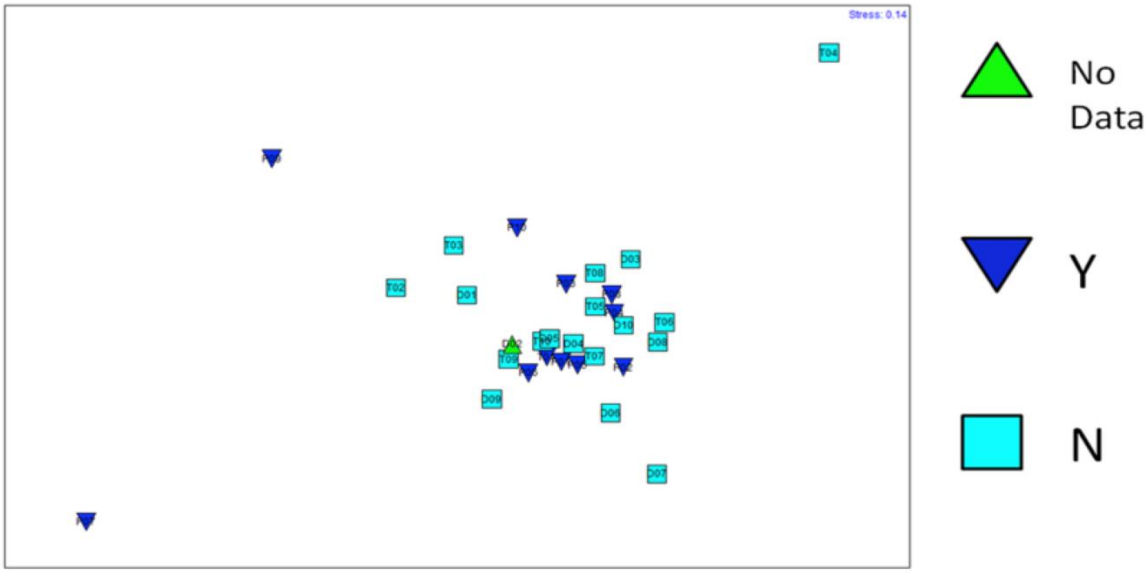

Figure B.6: MDS from bacterial fragments using presence/absence of PCBs based on a threshold of 450 ppb.

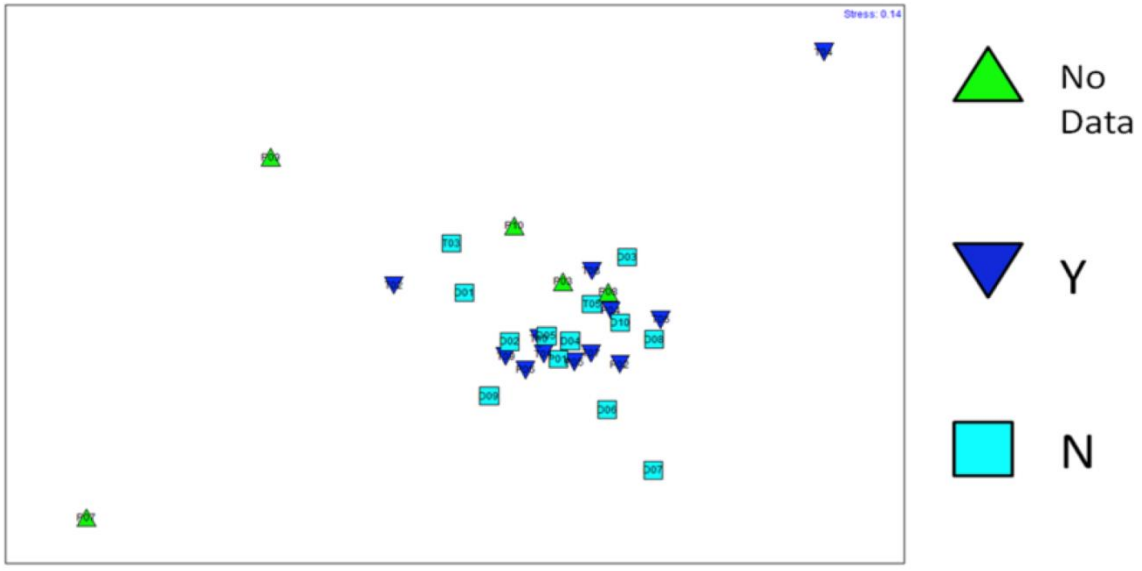

Figure B.7: MDS from bacterial fragments using presence/absence of Dioxin based on a threshold of 5 ppb.

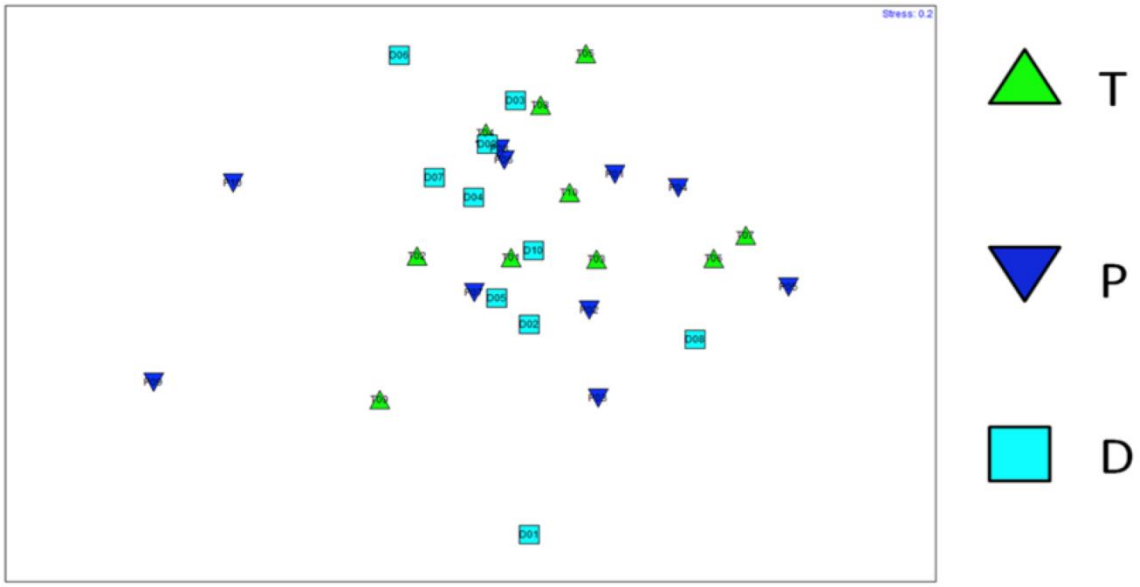

Figure B.8: MDS from fungal fragments using COI series as a factor.

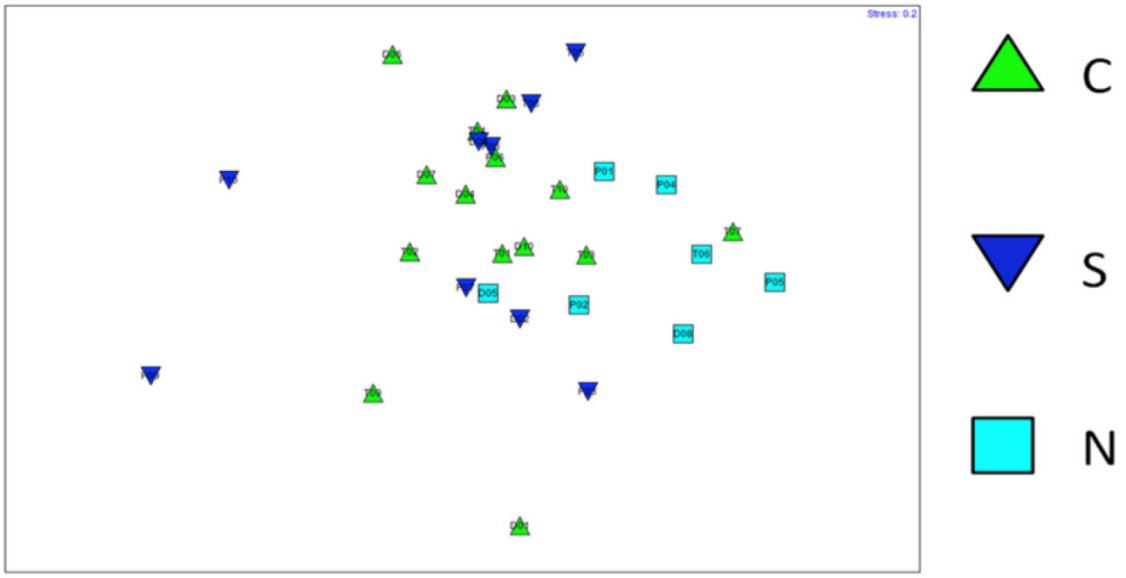

Figure B.9: MDS from fungal fragments with added factor for location based on the map in Figure 3.1. Sites were labeled south, central, and north.

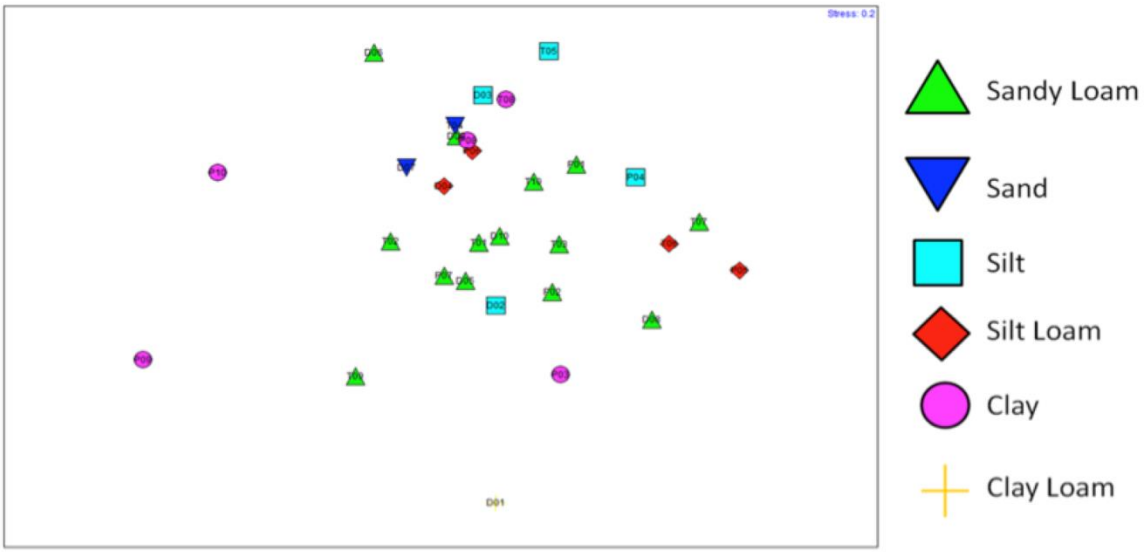

Figure B.10: MDS from fungal fragments with soil types

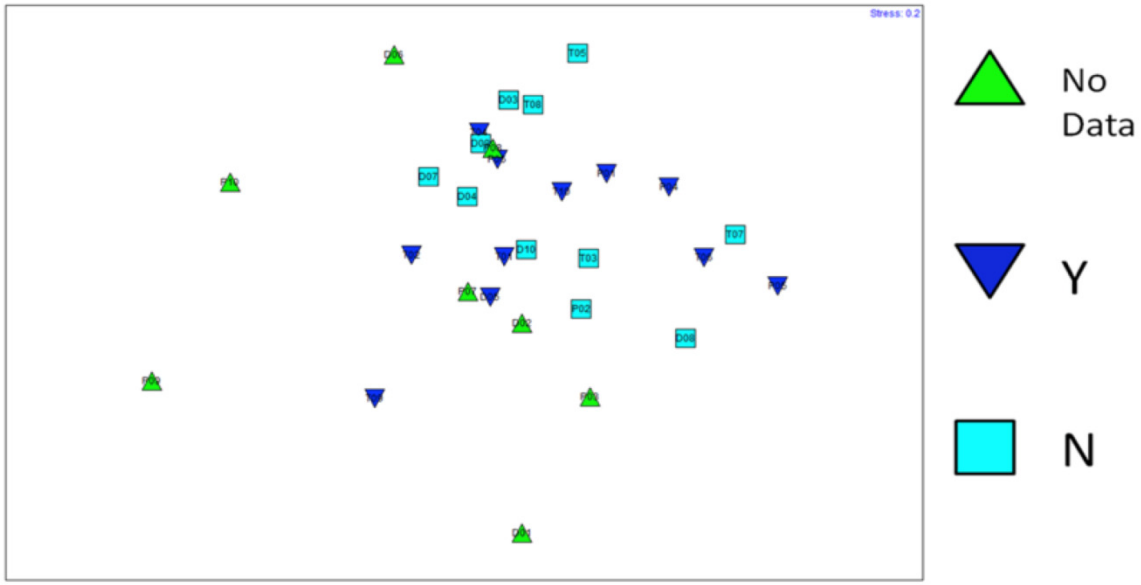

Figure B.11: MDS from fungal fragments using presence/absence of TPH based on a threshold of 300 ppm.

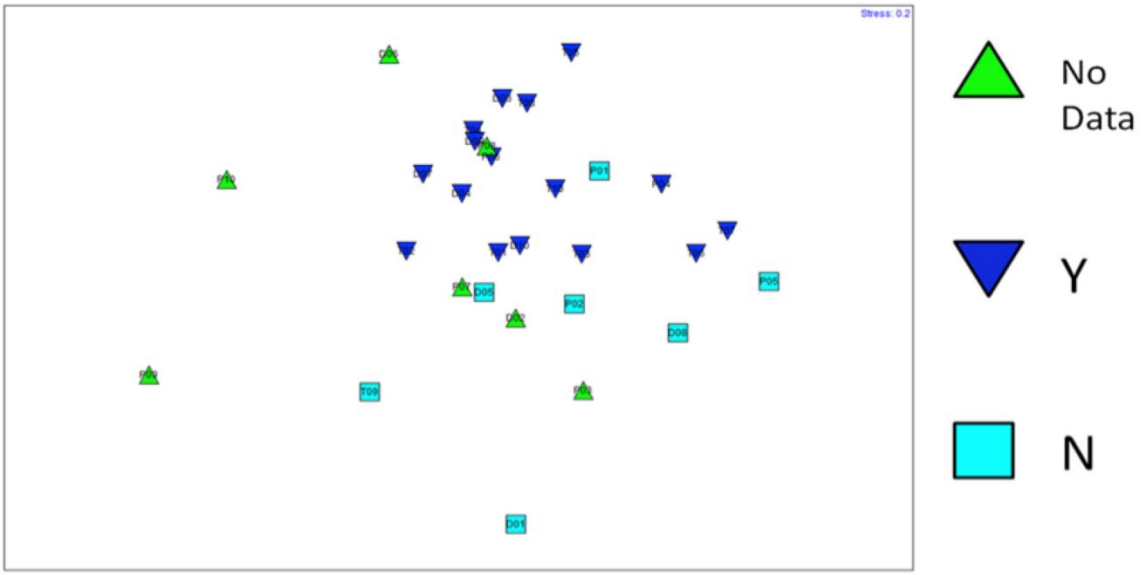

Figure B.12: MDS from fungal fragments using presence/absence of PAHs based on a threshold of 2.5 ppm.

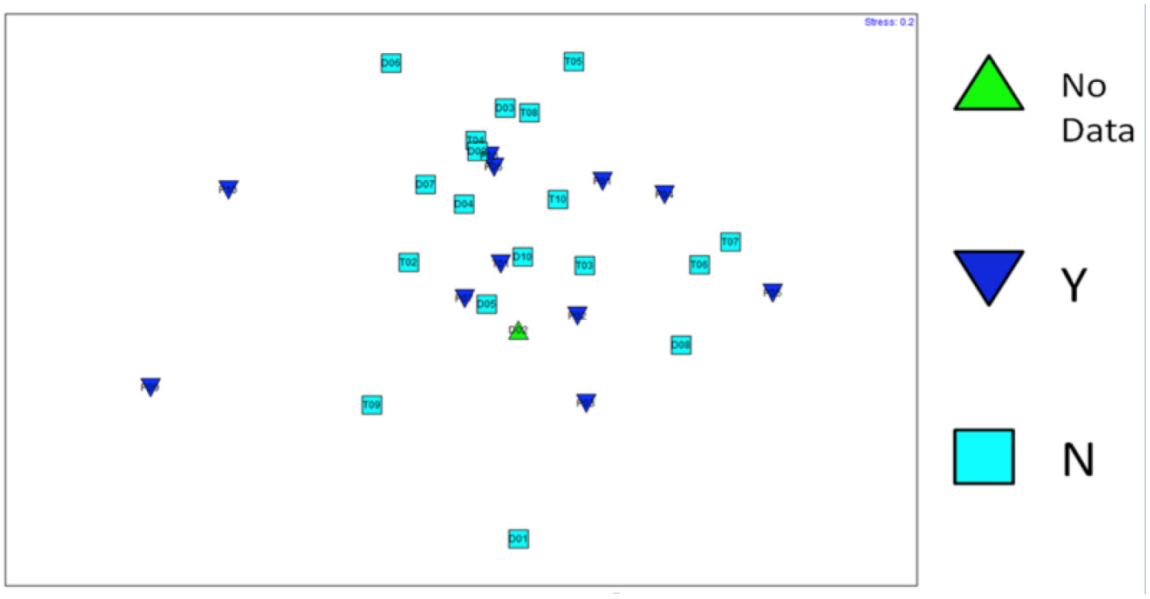

Figure B.13*:* MDS from fungal fragments using presence/absence of PCBs based on a threshold of 450 ppb.

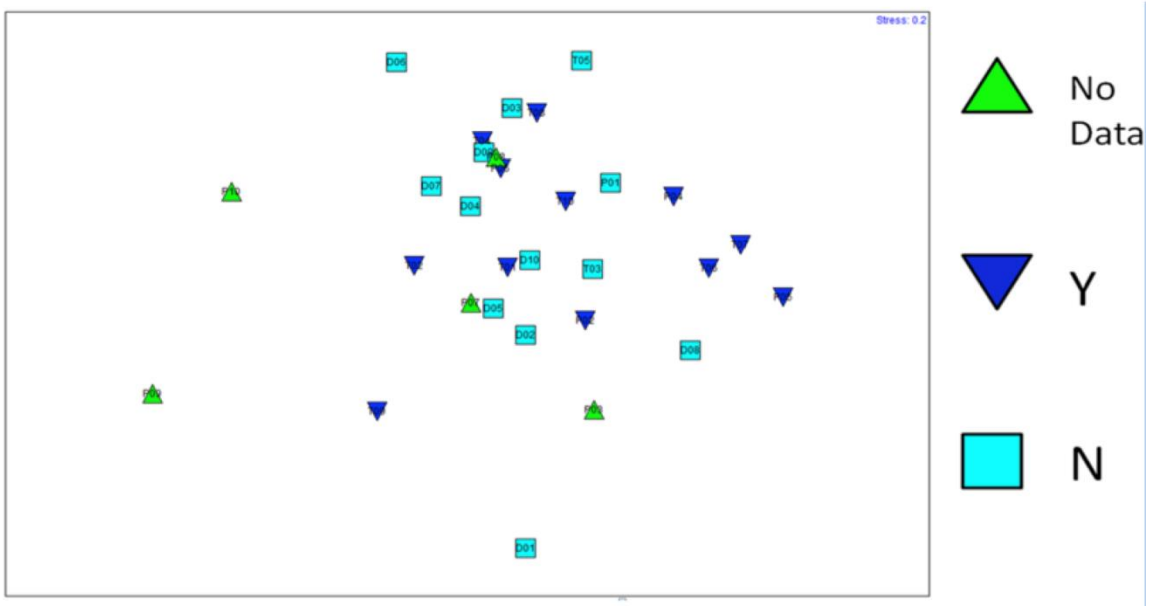

Figure B.14: MDS from fungal fragments using presence/absence of Dioxin based on a threshold of 5 ppb.

Appendix C: Taxonomic breakdown of five site soils from metagenomics analysis

(Samples D6.10, P4.10, T1.16, T6.16, and T7.16)

```
Taxonomy Summary for ./temp/D6-10/D6-10.RapTOR_2r

593453 reads placed to Division:
Eukaryota 3795
 Chordata 67 [1/1 to Order Rodentia]
 Fungi 8 (see below)
 Others 3720 [2458/3645 to Genus Trichomonas]
Bacteria 589121 (see below)
Archaea 16 [2/9 to Genus Desulfurococcus]
Eukaryotic viruses 0
Bacteriophages 0
Archaeal viruses 0
Other Sequences 521 [50/50 to Species synthetic construct]

Fungi, 8 reached Order: [8 to Genus]:
 Sordariales (Ascomycota) 8 [ 8] 8 Sordaria

Bacteria, 478403 reached Order: [265208 to Genus]:
 Actinomycetales (Actinobacteria) 101356 [ 92953] 24718 Mycobacterium
 18633 Streptomyces
 3655 Pseudonocardia
 Rhizobiales (Alphaproteobacteria) 62856 [ 49086] 9636 Rhodoplanes
 5619 Pleomorphomonas
 5433 Bradyrhizobium
 Gaiellales (Actinobacteria) 36809 [ 36809] 36809 Gaiella
 Rhodospirillales (Alphaproteobacteria) 24493 [ 22577] 5055 Nisaea
 4591 Tistrella
 4400 Dongia
 Solirubrobacterales (Actinobacteria) 22670 [ 22217] 20905 Solirubrobacter
 854 Conexibacter
 458 Patulibacter
 Acidobacteriales (Acidobacteria) 22284 [ 20018] 5454 Granulicella
 4496 Acidobacterium
 2705 Bryocella
 Other Orders 207935 [ 21548] 13570 Bacillus
 3397 Paenibacillus
 2192 Calditerricola
 389 Staphylococcus
 277 Kurthia
```

```
Taxonomy Summary for ./temp/P4-10/P4-10.RapTOR_2r

429749 reads placed to Division:
Eukaryota 7981
 Chordata 32 [1/3 to Order Anura]
 Fungi 25 (see below)
 Others 7924 [5177/7281 to Genus Trichomonas]
Bacteria 421664 (see below)
Archaea 21 [10/18 to Genus Thermococcus]
Eukaryotic viruses 0
Bacteriophages 0
Archaeal viruses 0
Other Sequences 83 [18/19 to Species synthetic construct]

Fungi, 25 reached Order: [24 to Genus]:
 Sordariales (Ascomycota) 24 [ 24] 24 Sordaria
 Saccharomycetales (Ascomycota) 1 [ 0]

Bacteria, 353046 reached Order: [191642 to Genus]:
 Actinomycetales (Actinobacteria) 82180 [ 74796] 7749 Streptomyces
 7196 Blastococcus
 7016 Arthrobacter
 Rhizobiales (Alphaproteobacteria) 44774 [ 40544] 11208 Methylobacterium
 4743 Bradyrhizobium
 3646 Microvirga
 Burkholderiales (Betaproteobacteria) 22751 [ 17008] 2731 Massilia
 2579 Herbaspirillum
 2571 Burkholderia
 Sphingomonadales (Alphaproteobacteria) 19686 [ 17873] 11874 Sphingomonas
 1662 Novosphingobium
 1441 Kaistobacter
 Sphingobacteriales (Bacteroidetes) 15249 [ 14536] 4924 Segetibacter
 2691 Flavisolibacter
 2113 Chitinophaga
 Rhodospirillales (Alphaproteobacteria) 14189 [ 13192] 4110 Azospirillum
 2178 Skermanella
 1590 Roseomonas
 Other Orders 154217 [ 13693] 13693 Gaiella
```

```
Taxonomy Summary for ./temp/P4-10/P4-10.RapTOR_2r

429749 reads placed to Division:
Eukaryota 7981
 Chordata 32 [1/3 to Order Anura]
 Fungi 25 (see below)
 Others 7924 [5177/7281 to Genus Trichomonas]
Bacteria 421664 (see below)
Archaea 21 [10/18 to Genus Thermococcus]
Eukaryotic viruses 0
Bacteriophages 0
Archaeal viruses 0
Other Sequences 83 [18/19 to Species synthetic construct]

Fungi, 25 reached Order: [24 to Genus]:
 Sordariales (Ascomycota) 24 [ 24] 24 Sordaria
 Saccharomycetales (Ascomycota) 1 [ 0]

Bacteria, 353046 reached Order: [191642 to Genus]:
 Actinomycetales (Actinobacteria) 82180 [ 74796] 7749 Streptomyces
 7196 Blastococcus
 7016 Arthrobacter
 Rhizobiales (Alphaproteobacteria) 44774 [ 40544] 11208 Methylobacterium
 4743 Bradyrhizobium
 3646 Microvirga
 Burkholderiales (Betaproteobacteria) 22751 [ 17008] 2731 Massilia
 2579 Herbaspirillum
 2571 Burkholderia
 Sphingomonadales (Alphaproteobacteria) 19686 [ 17873] 11874 Sphingomonas
 1662 Novosphingobium
 1441 Kaistobacter
 Sphingobacteriales (Bacteroidetes) 15249 [ 14536] 4924 Segetibacter
 2691 Flavisolibacter
 2113 Chitinophaga
 Rhodospirillales (Alphaproteobacteria) 14189 [ 13192] 4110 Azospirillum
 2178 Skermanella
 1590 Roseomonas
 Other Orders 154217 [ 13693] 13693 Gaiella
```

```
Taxonomy Summary for ./temp/T6-16/T6-16.RapTOR_2r

1013040 reads placed to Division:
Eukaryota 8328
 Chordata 379 [4/5 to Order Rodentia]
 Fungi 14 (see below)
 Others 7935 [3415/7622 to Genus Trichomonas]
Bacteria 1004268 (see below)
Archaea 81 [12/43 to Genus Thermogladius]
Eukaryotic viruses 0
Bacteriophages 0
Archaeal viruses 0
Other Sequences 363 [44/45 to Species synthetic construct]

Fungi, 14 reached Order: [14 to Genus]:
 Sordariales (Ascomycota) 14 [ 14] 14 Sordaria

Bacteria, 842610 reached Order: [554850 to Genus]:
 Actinomycetales (Actinobacteria) 209293 [195040] 45076 Streptomyces
 21172 Pseudonocardia
 13902 Kribbella
 Gaiellales (Actinobacteria) 172397 [172397] 172397 Gaiella
 Rhizobiales (Alphaproteobacteria) 82998 [ 73445] 25474 Bradyrhizobium
 14541 Rhodoplanes
 5978 Methylobacterium
 Solirubrobacterales (Actinobacteria) 33725 [ 33037] 29969 Solirubrobacter
 1883 Conexibacter
 1185 Patulibacter
 Rhodospirillales (Alphaproteobacteria) 30982 [ 28860] 11510 Azospirillum
 4148 Skermanella
 1737 Roseomonas
 Myxococcales (Deltaproteobacteria) 28734 [ 26759] 14128 Sorangium
 2947 Chondromyces
 2456 Anaeromyxobacter
 Other Orders 284481 [ 25312] 18254 Bacillus
 1987 Paenibacillus
 560 Staphylococcus
 545 Shimazuella
 452 Thermoflavimicrobium
```

```
Taxonomy Summary for ./temp/T7-16/T7-16.RapTOR_2r

1426612 reads placed to Division:
Eukaryota 9967
 Chordata 80 [2/3 to Order Rodentia]
 Fungi 26 (see below)
 Others 9861 [6505/9677 to Genus Trichomonas]
Bacteria 1415492 (see below)
Archaea 181 [50/120 to Genus Sulfophobococcus]
Eukaryotic viruses 0
Bacteriophages 0
Archaeal viruses 0
Other Sequences 972 [108/109 to Species synthetic construct]

Fungi, 26 reached Order: [26 to Genus]:
 Sordariales (Ascomycota) 26 [ 26] 26 Sordaria

Bacteria, 1155824 reached Order: [631633 to Genus]:
 Actinomycetales (Actinobacteria) 265199 [247304] 60153 Streptomyces
 21049 Gordonia
 17914 Pseudonocardia
 Gaiellales (Actinobacteria) 129570 [129570] 129570 Gaiella
 Rhizobiales (Alphaproteobacteria) 85309 [ 70003] 19487 Bradyrhizobium
 7466 Rhodoplanes
 7189 Methylobacterium
 Bacillales (Firmicutes) 68389 [ 66316] 50129 Bacillus
 5571 Paenibacillus
 2083 Ammoniphilus
 Solirubrobacterales (Actinobacteria) 46132 [ 45275] 42132 Solirubrobacter
 2247 Conexibacter
 896 Patulibacter
 Myxococcales (Deltaproteobacteria) 41283 [ 39017] 12322 Haliangium
 9294 Sorangium
 4129 Jahnella
 Other Orders 519942 [ 34148] 11088 Burkholderia
 6498 Ralstonia
 4107 Derxia
 3062 Sutterella
 2189 Ottowia
```

Appendix D: Microcosm results for EFH Equivalent Carbon Ranges

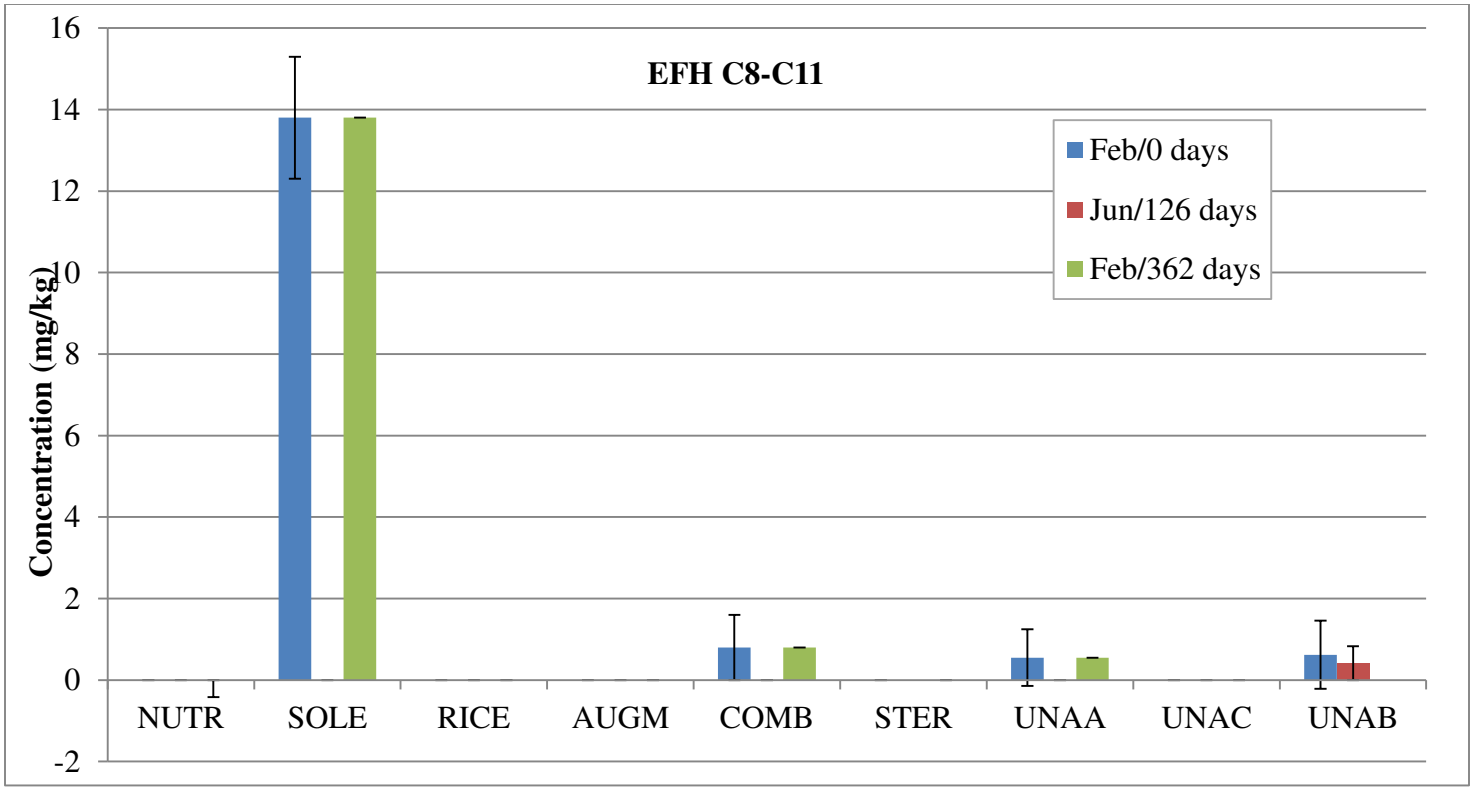

Figure D-1: C8-C11 EFH equivalent carbon ranges during microcosm incubation

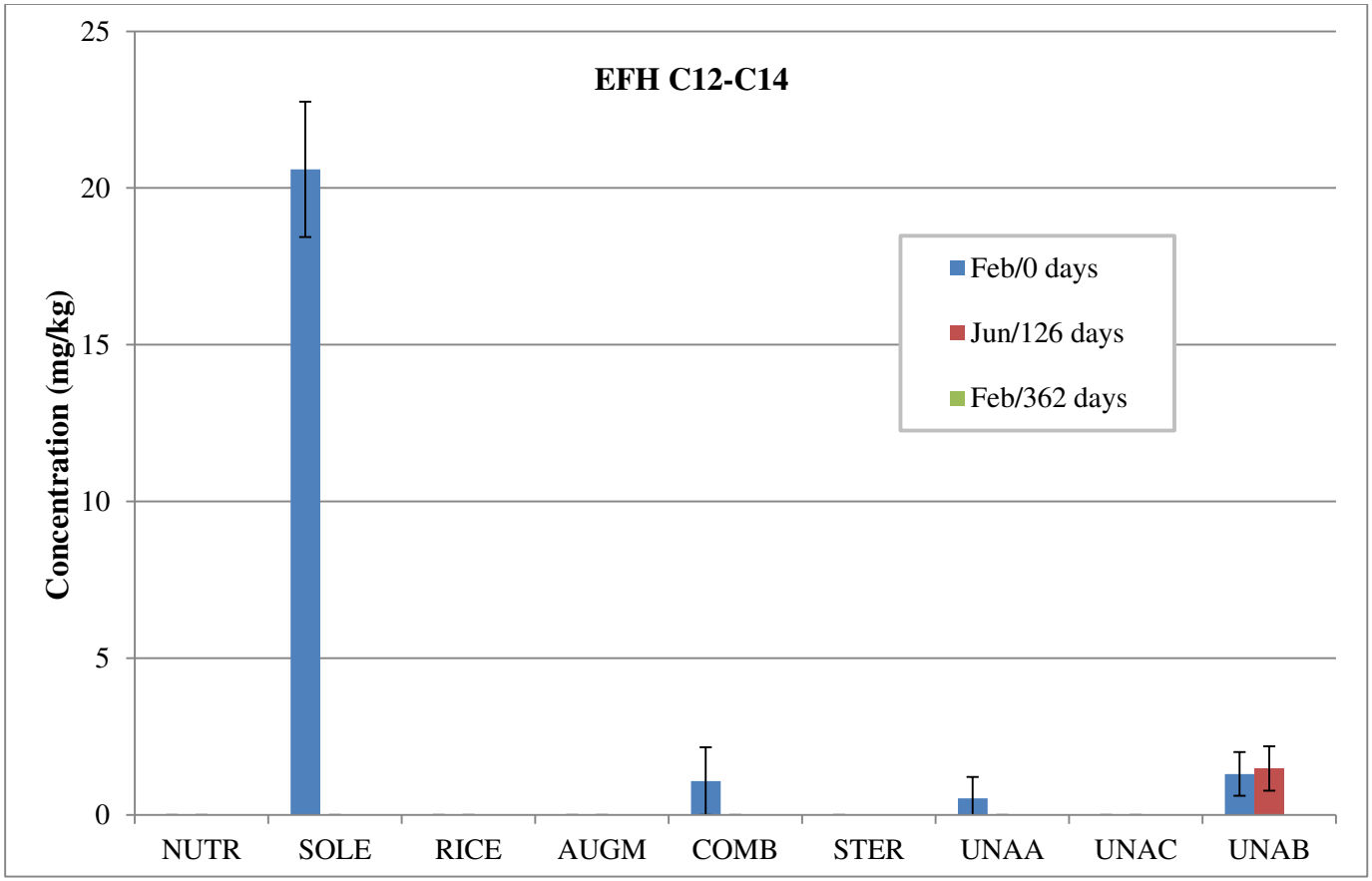

Figure D-2: EFH C12-C14 concentrations during microcosm incubation

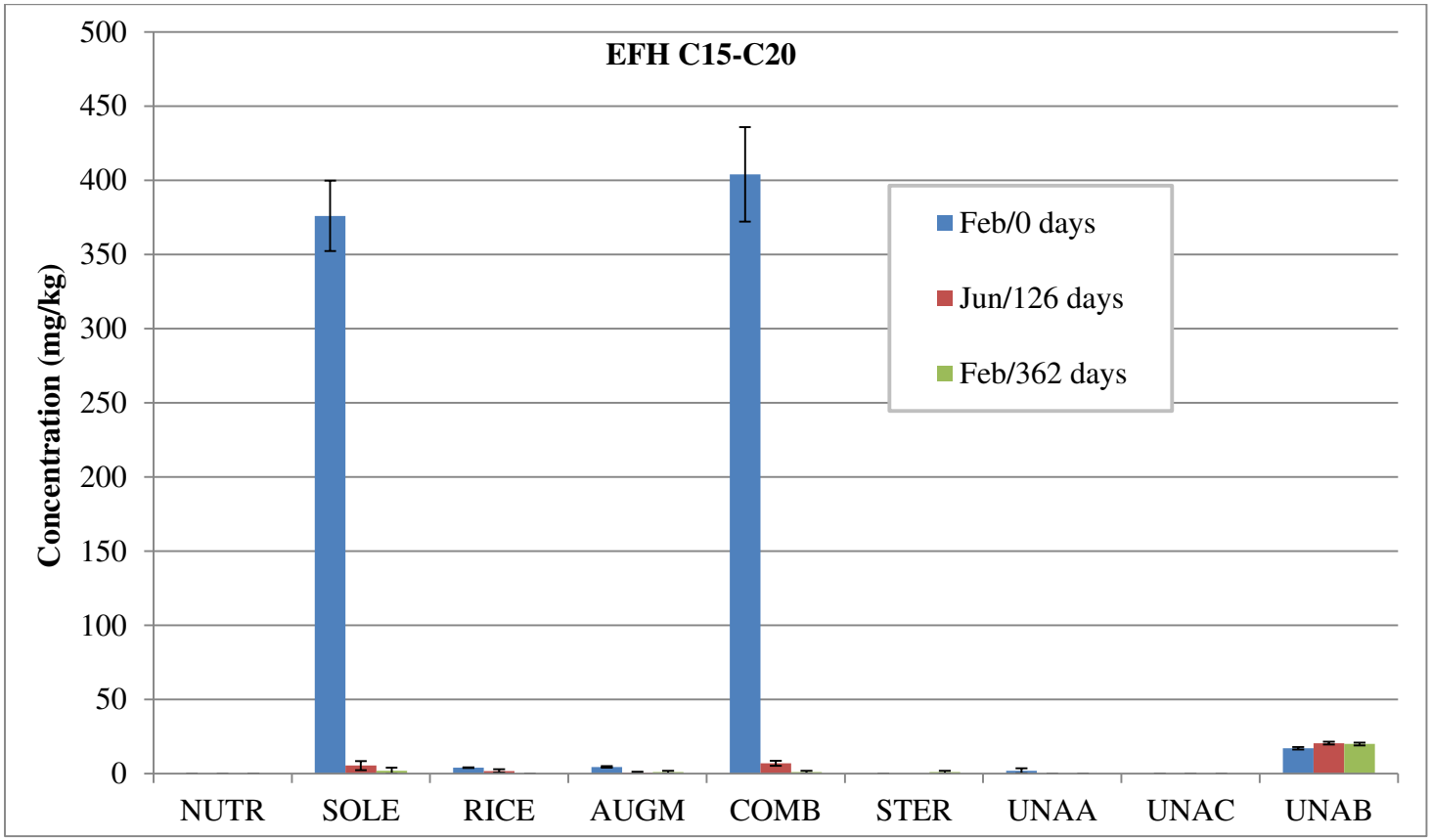

Figure D-3: EFH C15-C20 concentrations during microcosm incubation

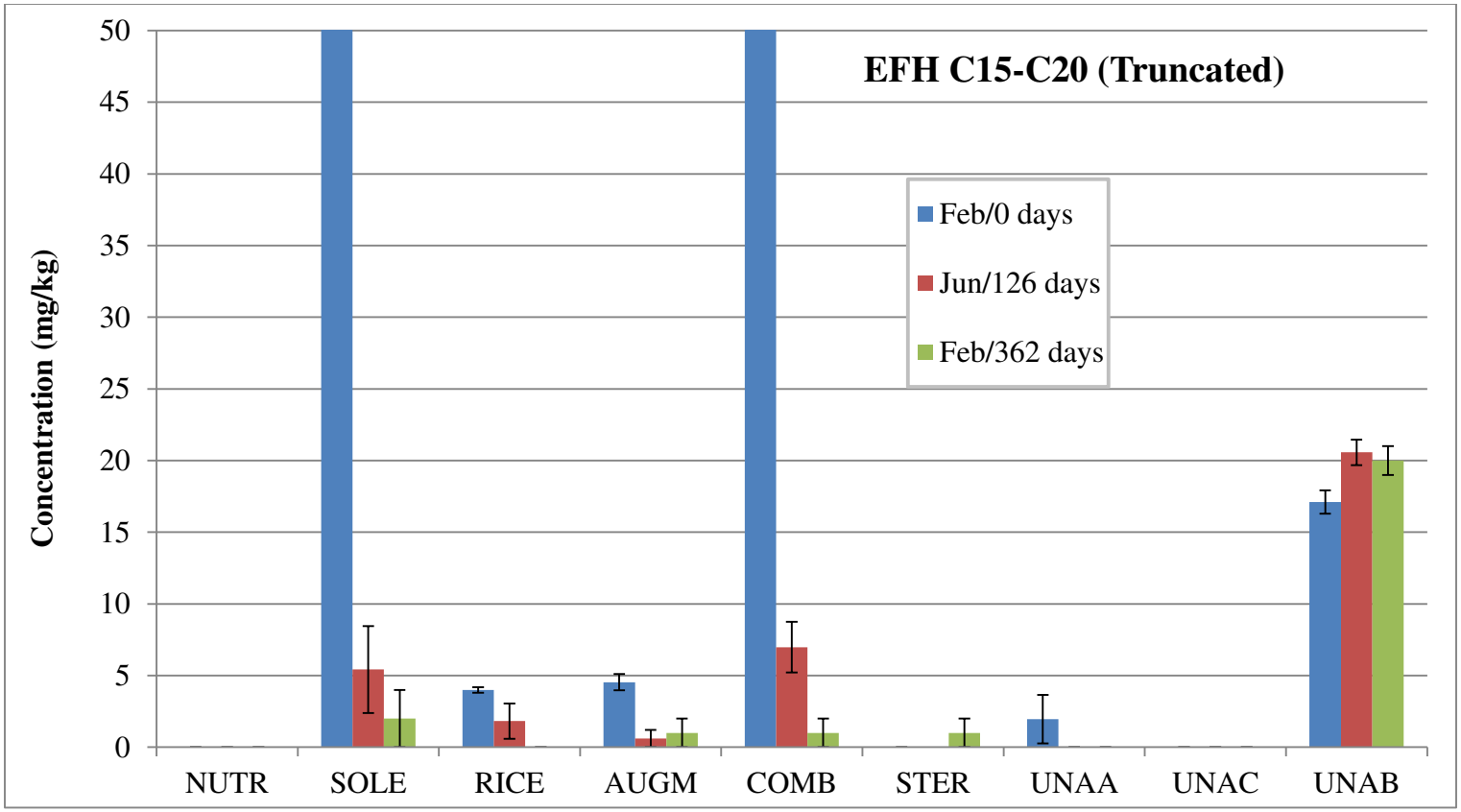

Figure D-4: Truncated EFH C15-C20 concentrations during microcosm incubation

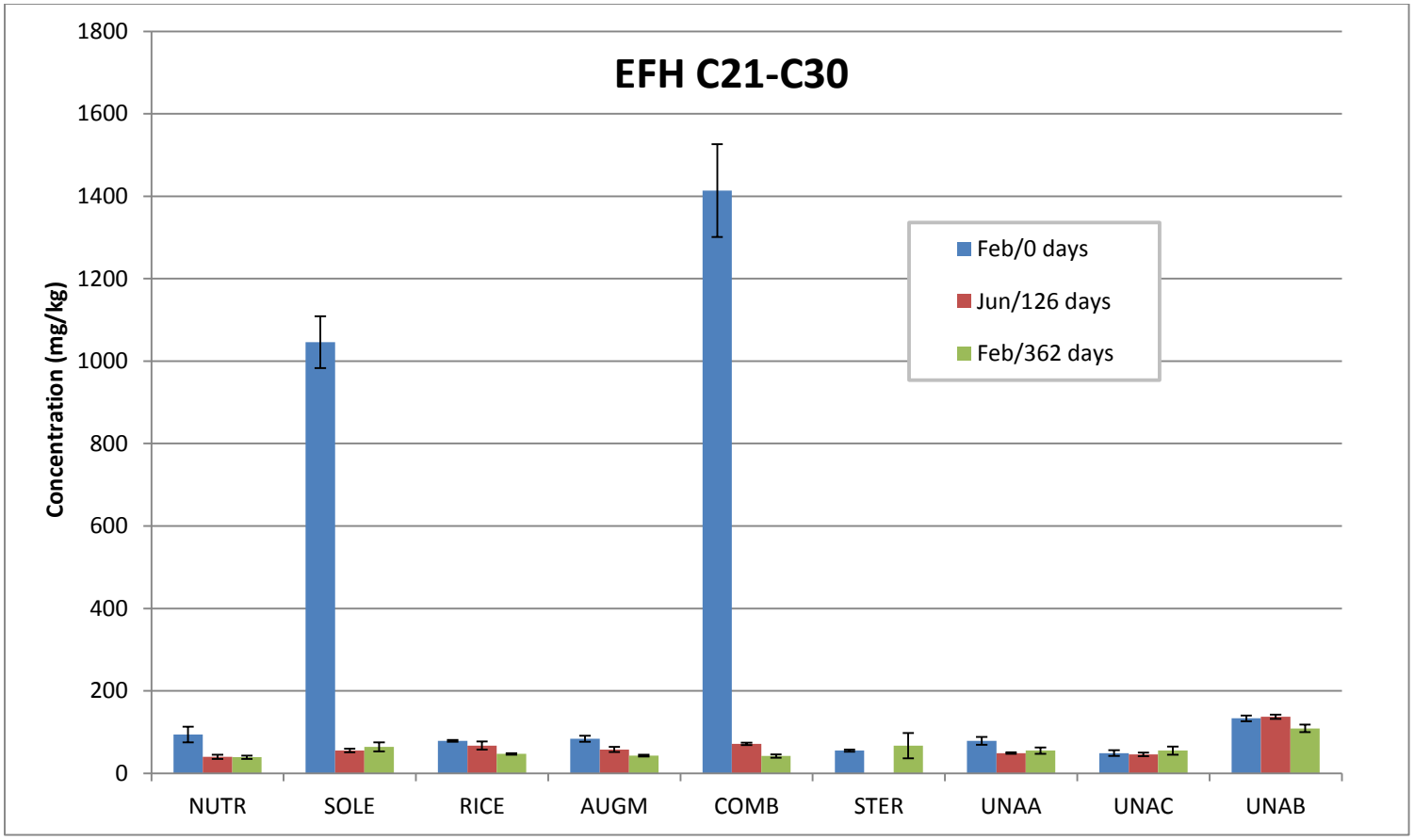

Figure D-5: EFH C21-C30 concentrations during microcosm incubation

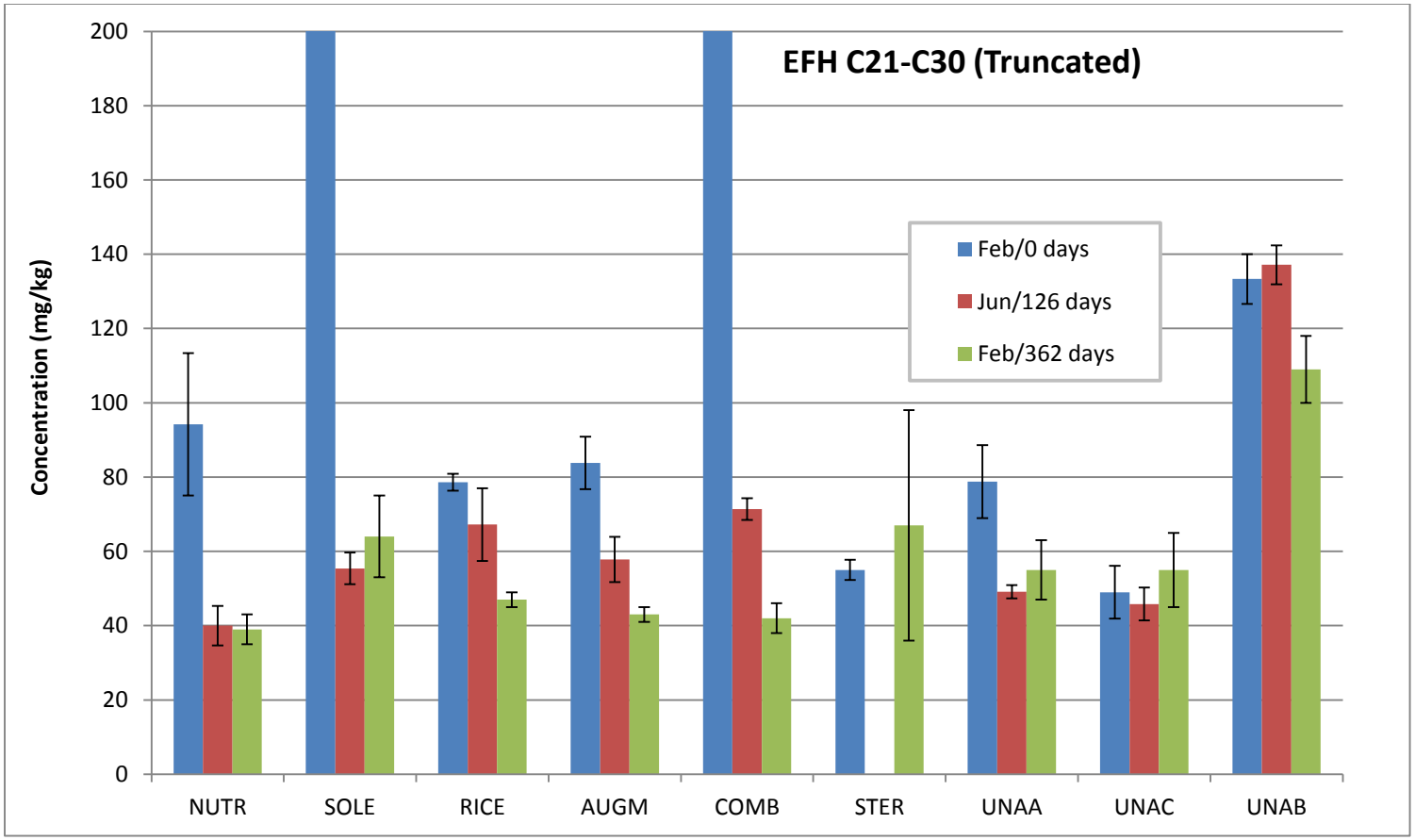

Figure D-6: Truncated EFH C21-C30 concentrations during microcoms incubation

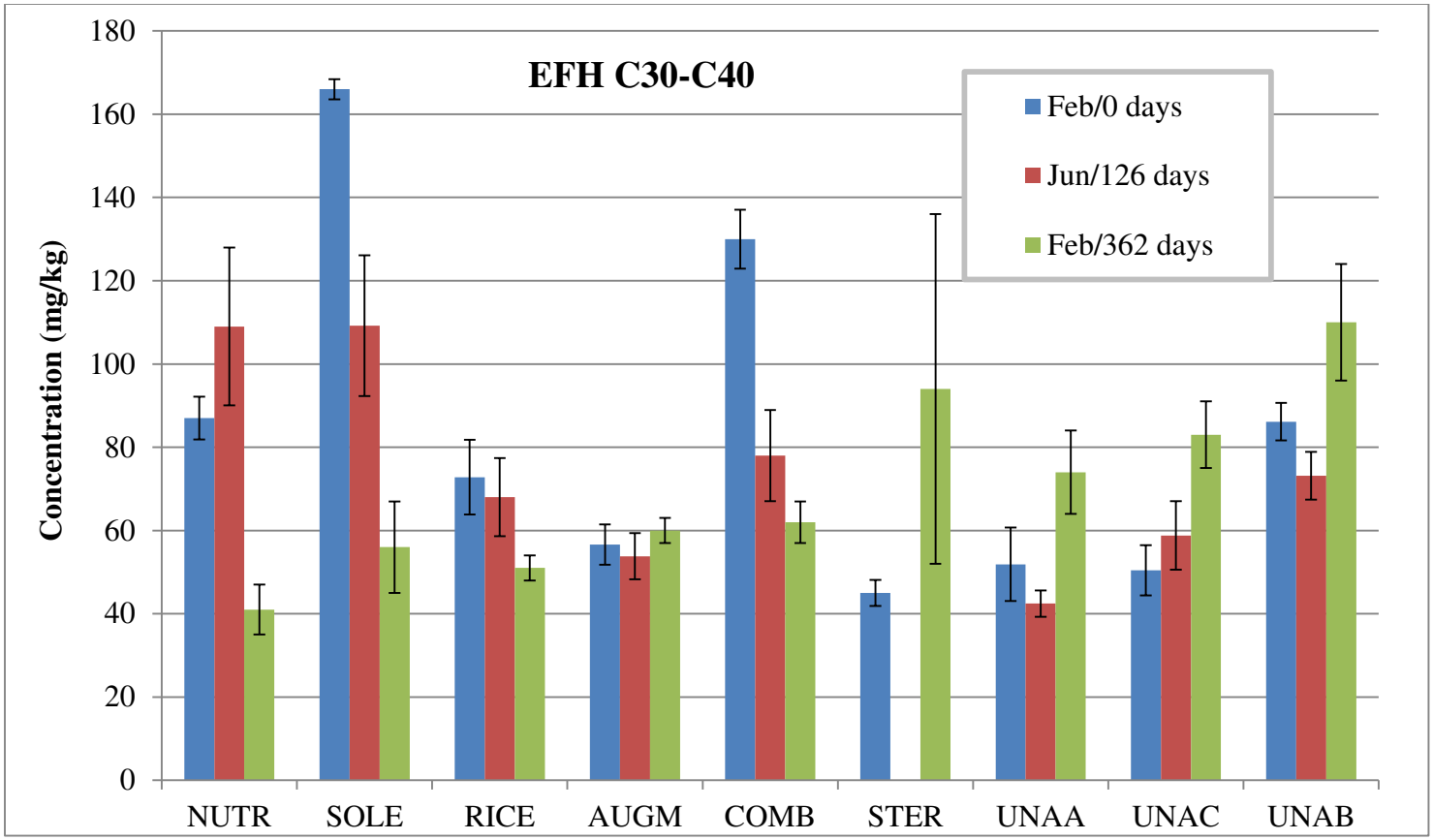

Figure D-7: EFH C30-C40 concentrations during microcosm incubation

Appendix E: Microcosm Results for Individual Chlorinated Dioxin Congeners

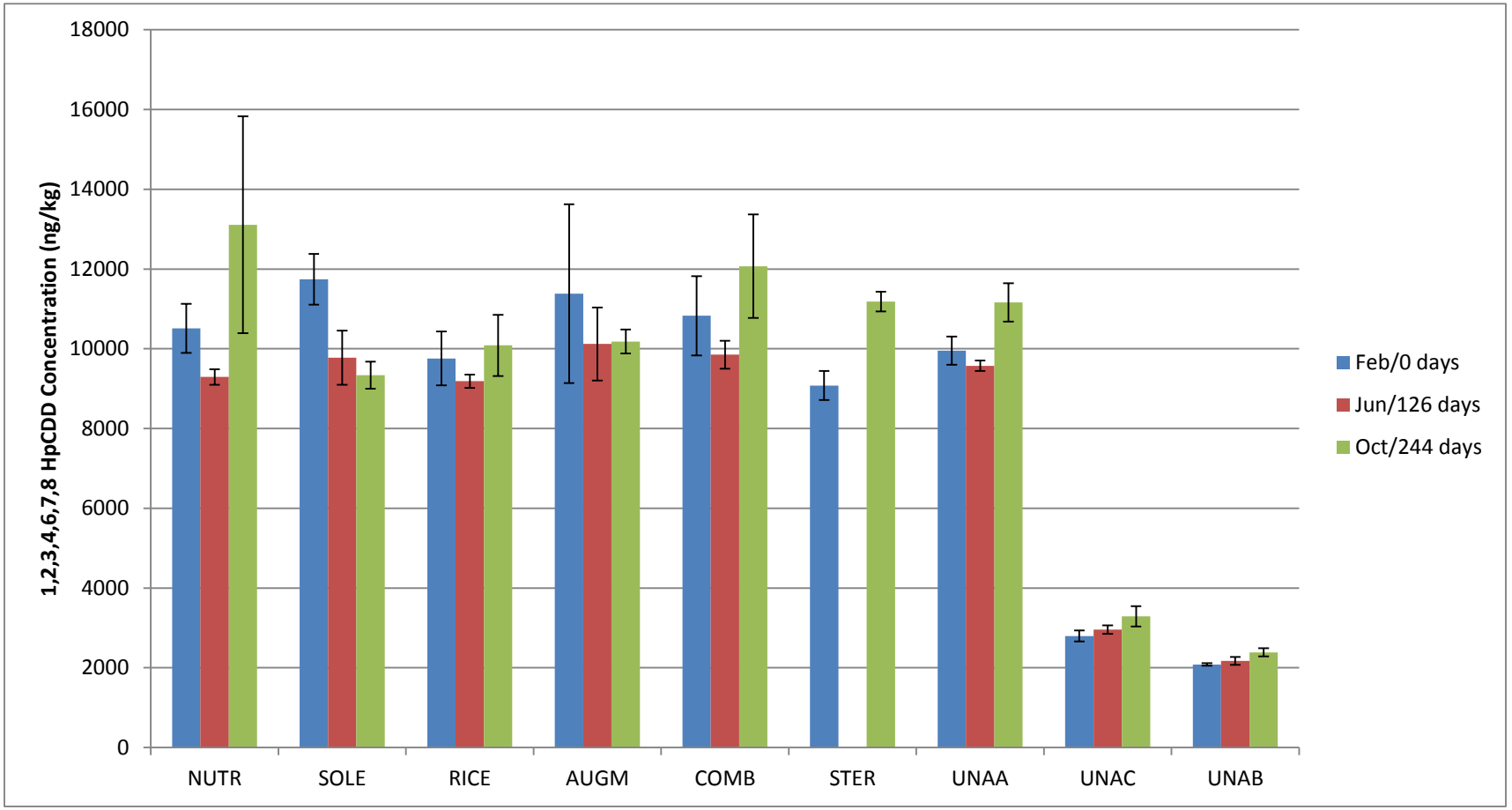

Figure E-1: 1,2,3,4,6,7,8 HpCDD concentrations during microcosm incubation

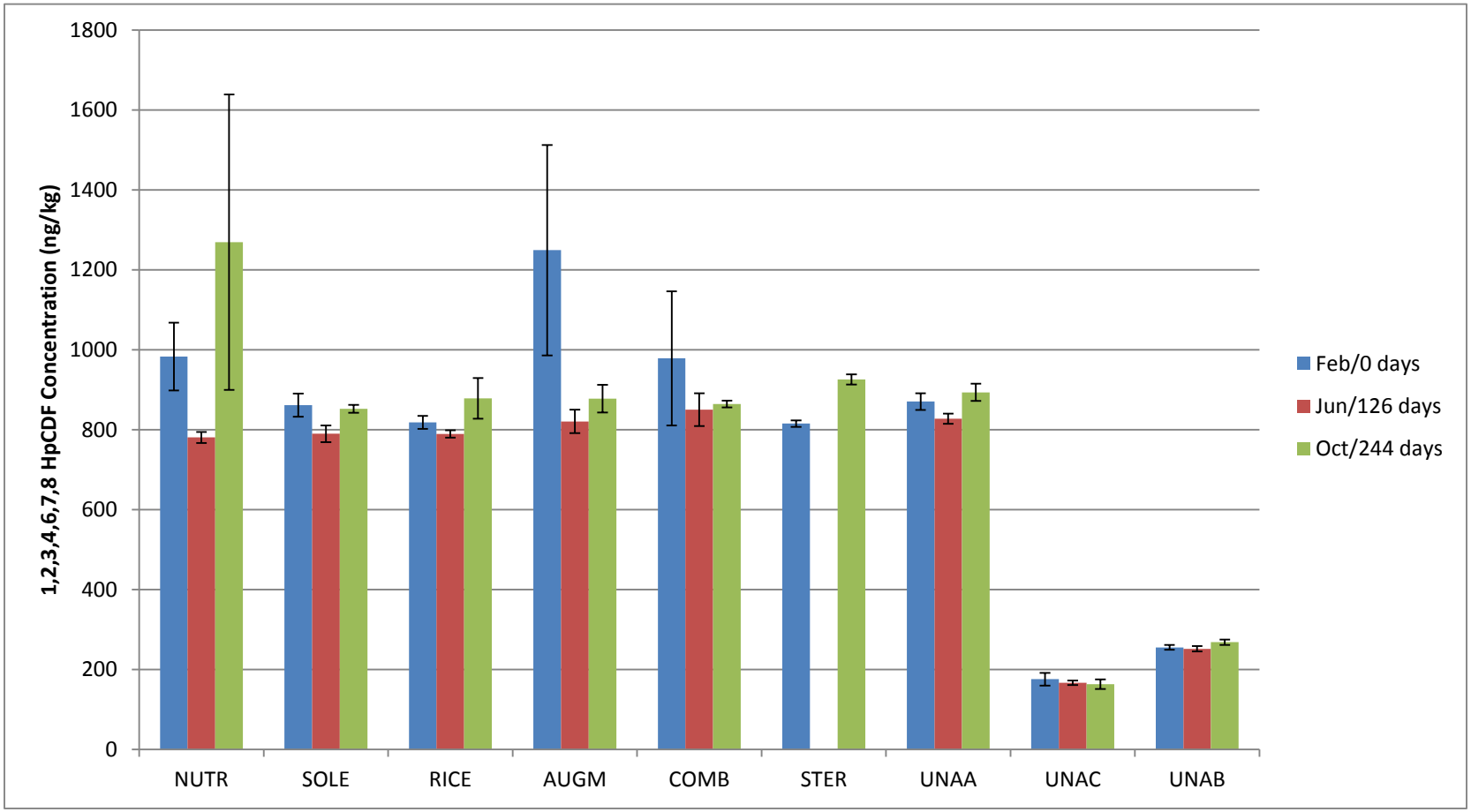

Figure E-2: 1,2,3,4,7,8, HpCDF concentrations during microcosm incubation

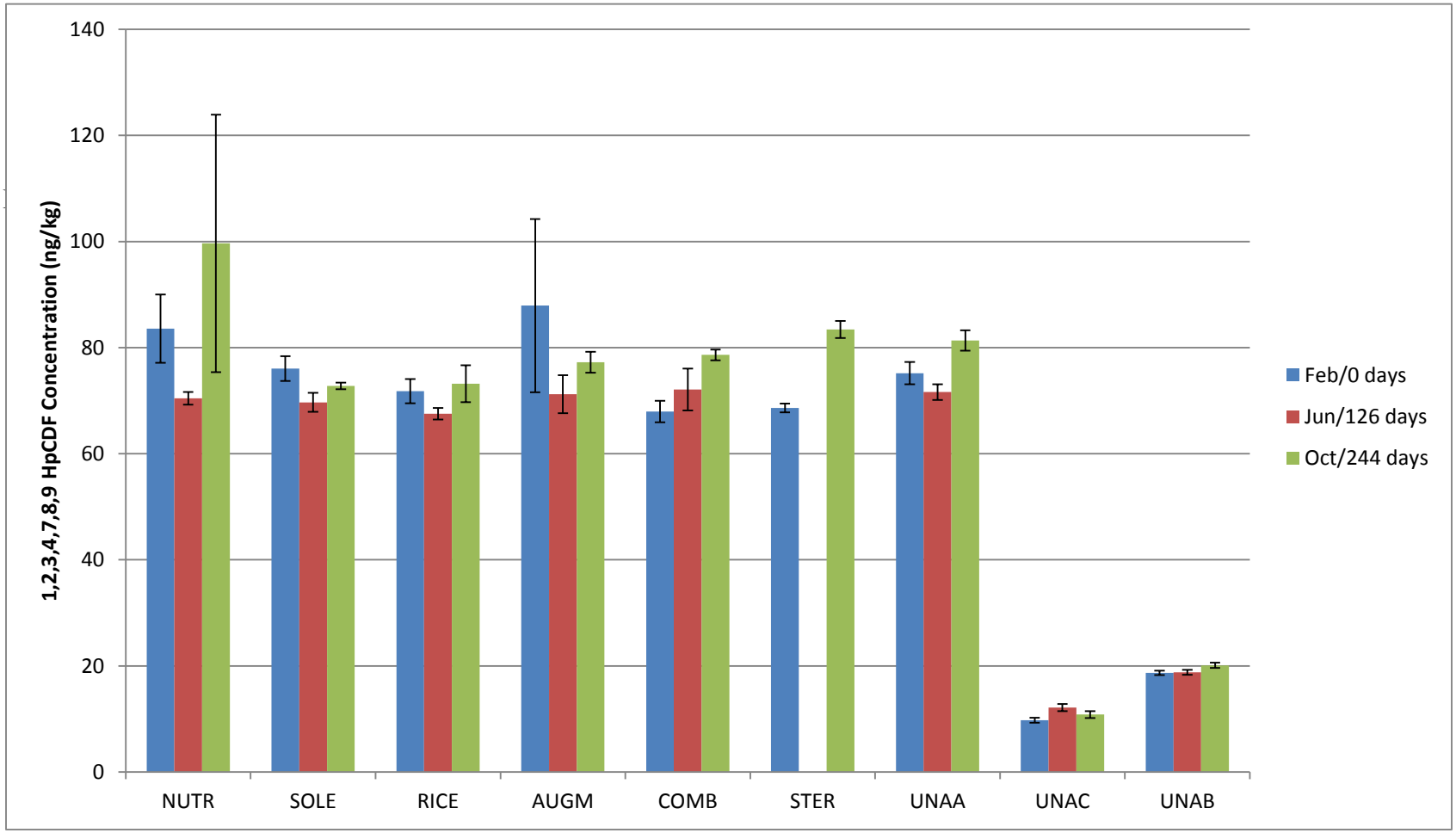

Figure E-3: 1,2,3,4,7,8,9 HpCDF concentrations during microcosm incubation

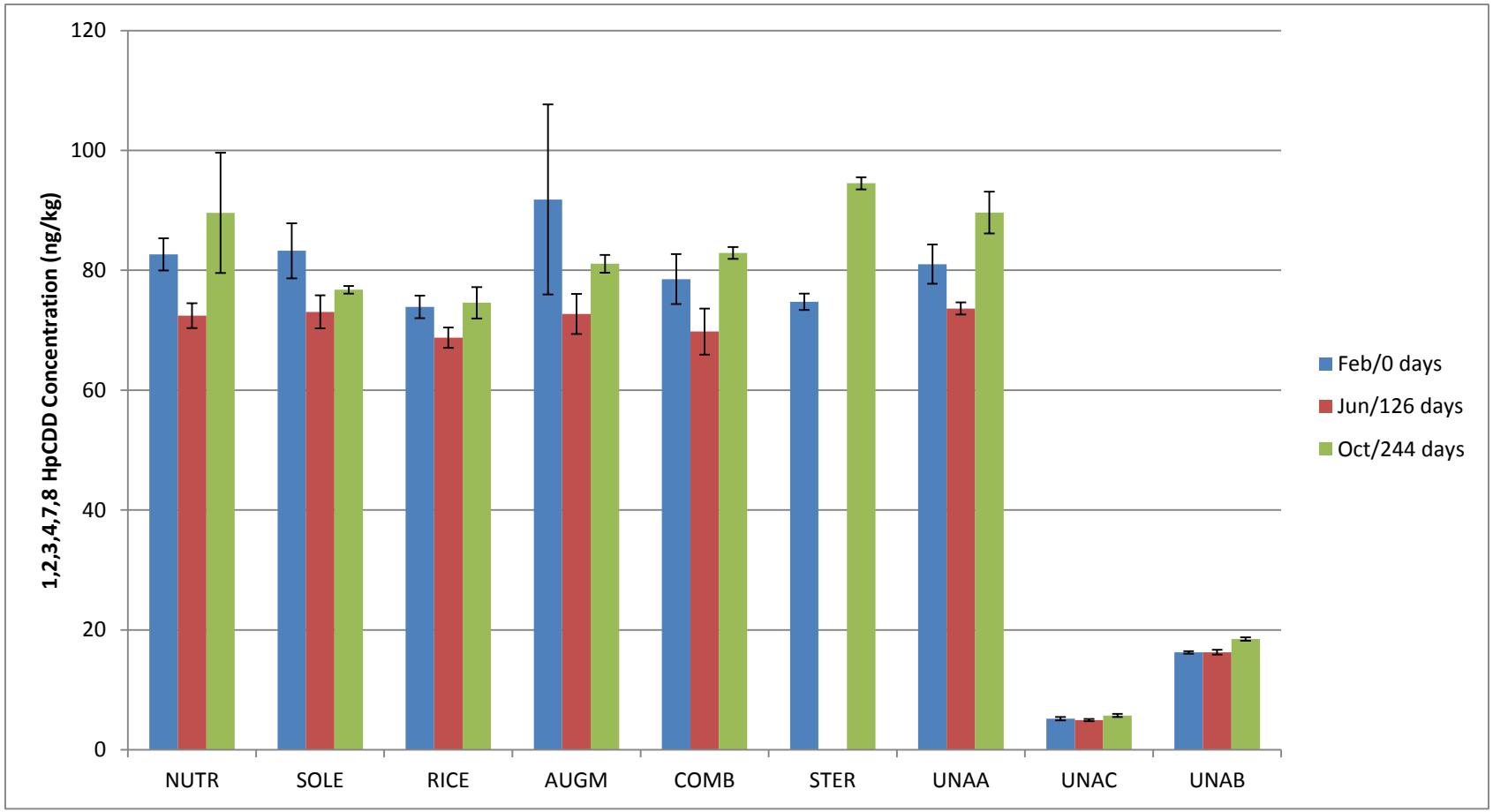

Figure E-4: 1,2,3,4,7,8 HpCDD concentrations during microcosm incubation

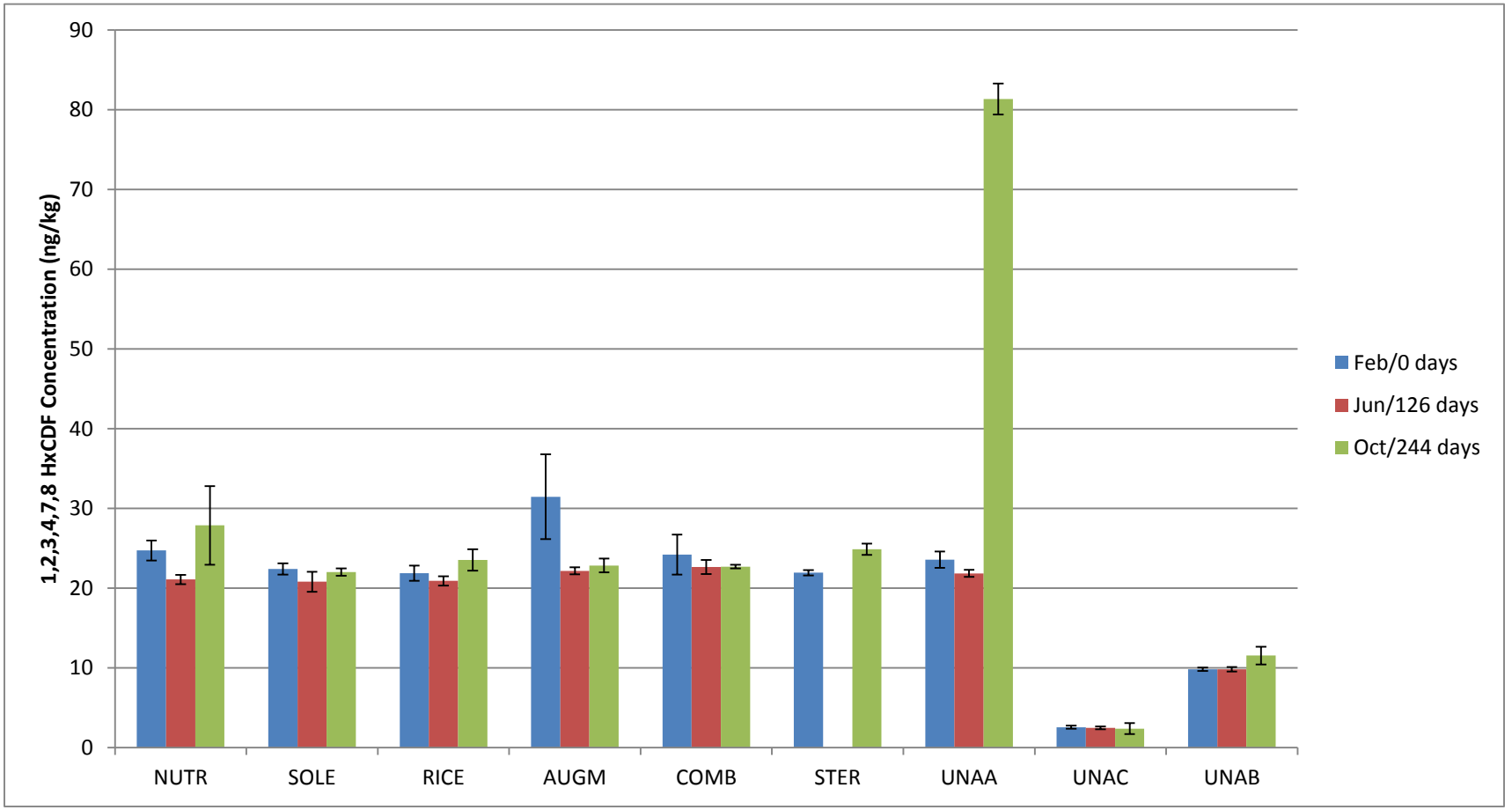

Figure E-5: 1,2,3,4,7,8 HxCDF concentrations during microcosm incubation

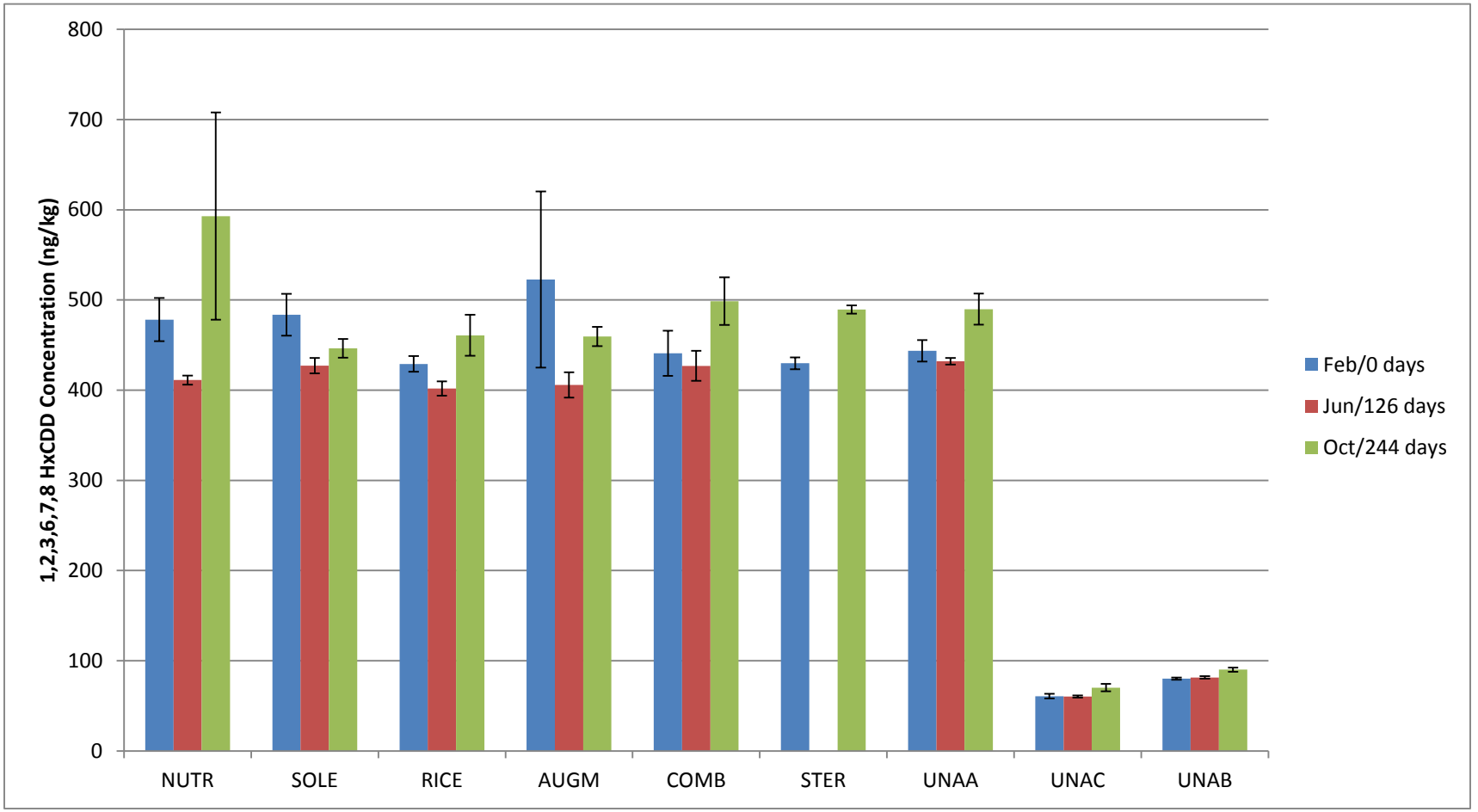

Figure E-6: 1,2,3,6,7,8 HxCDD concentrations during microcosm incubation

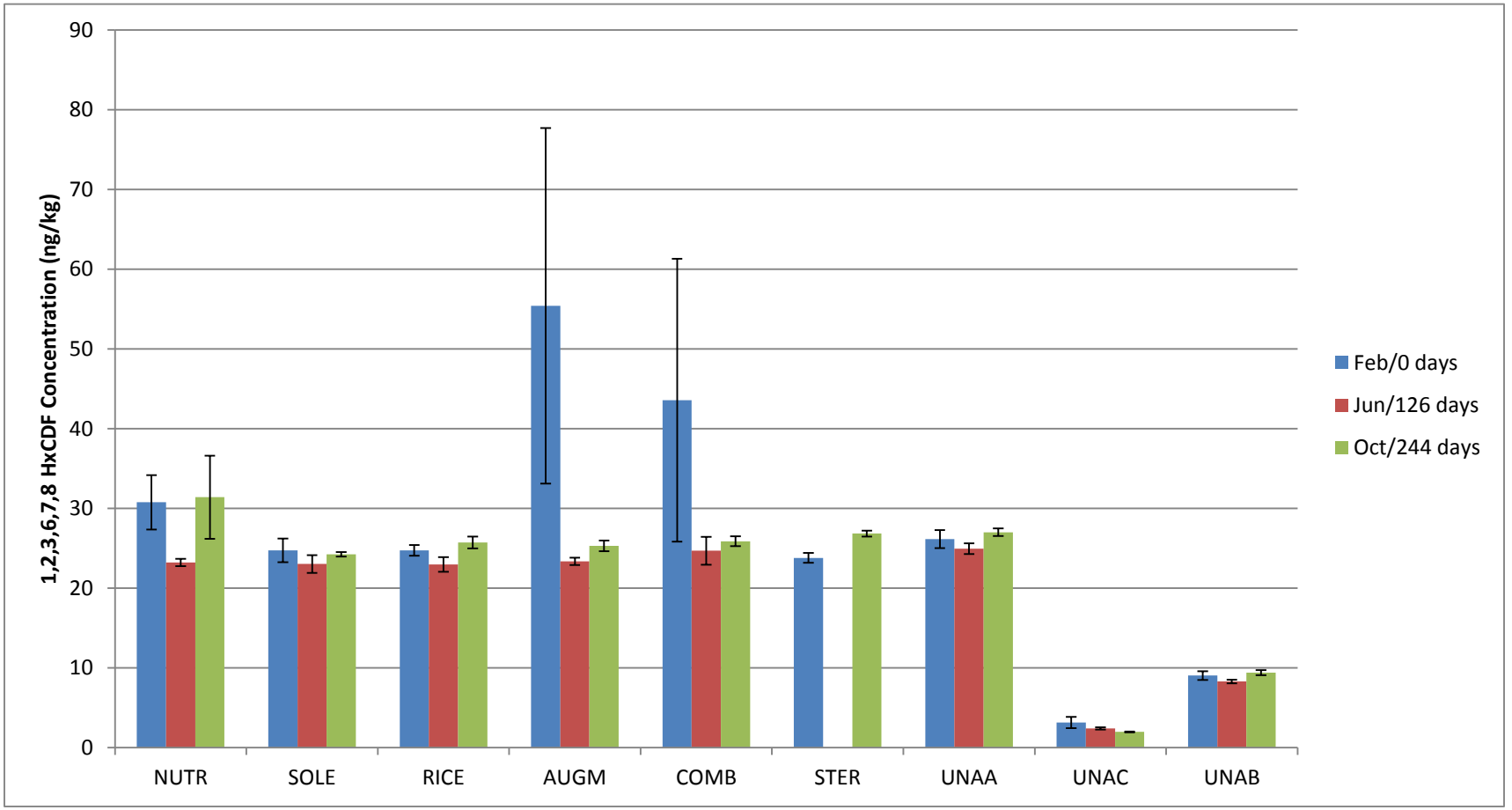

Figure E-7: 1,2,3,6,7,8 HxCDF concentrations during microcosm incubation

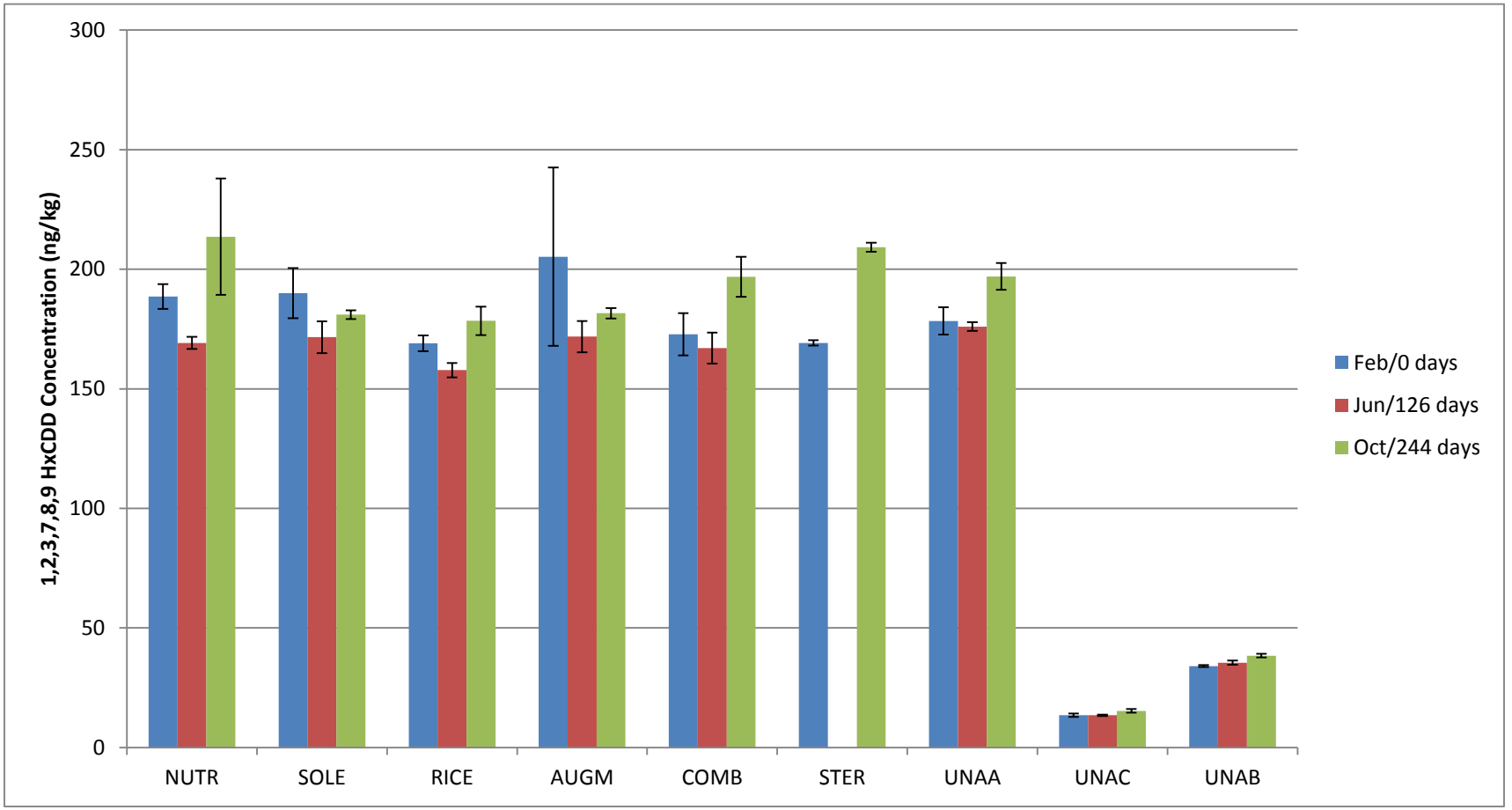

Figure E-8: 1,2,3,7,8,9 HxCDD concentrations during microcosm incubation

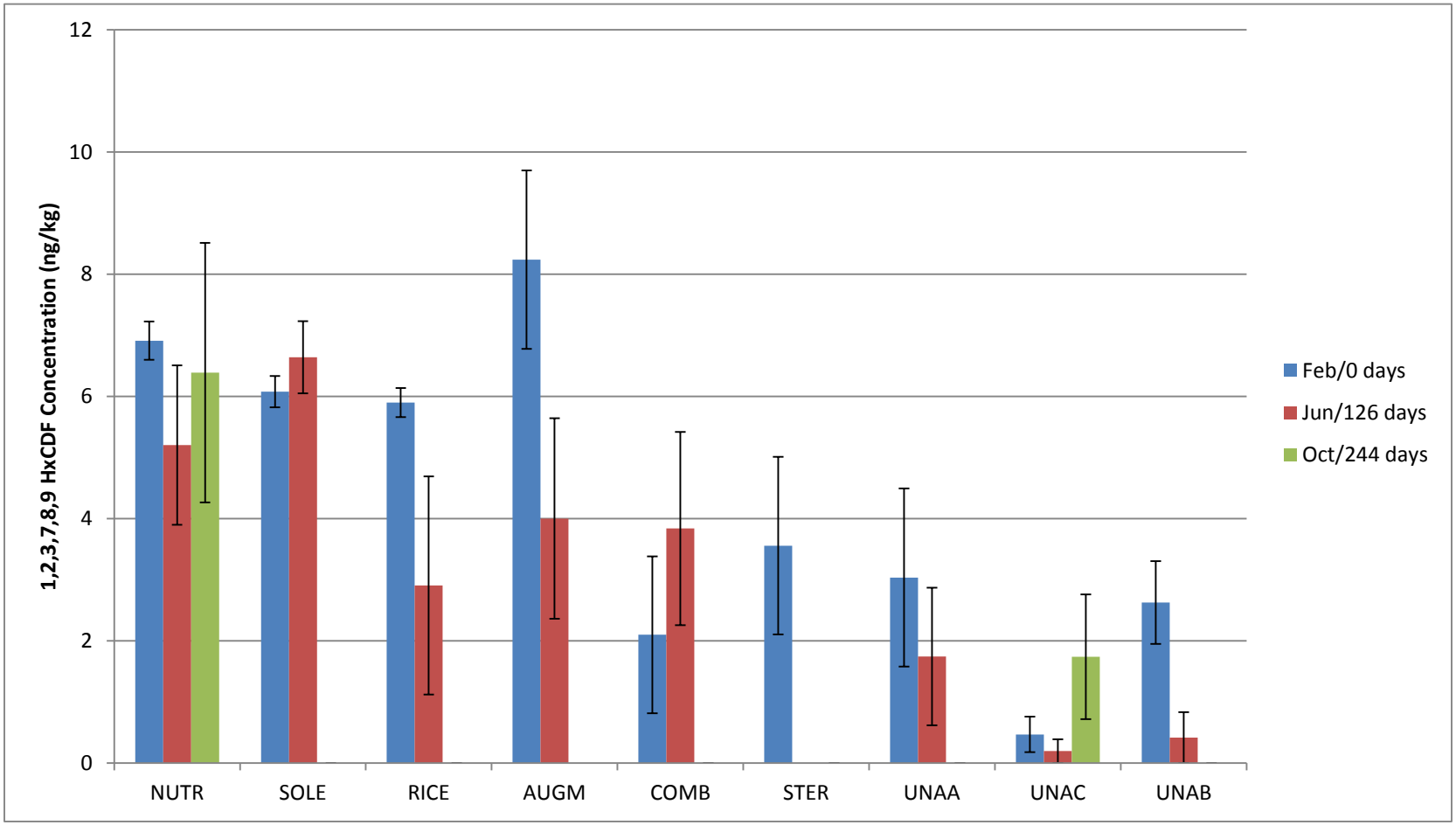

Figure E-9: 1,2,3,7,8,9 HxCDF concentrations during microcosm incubation

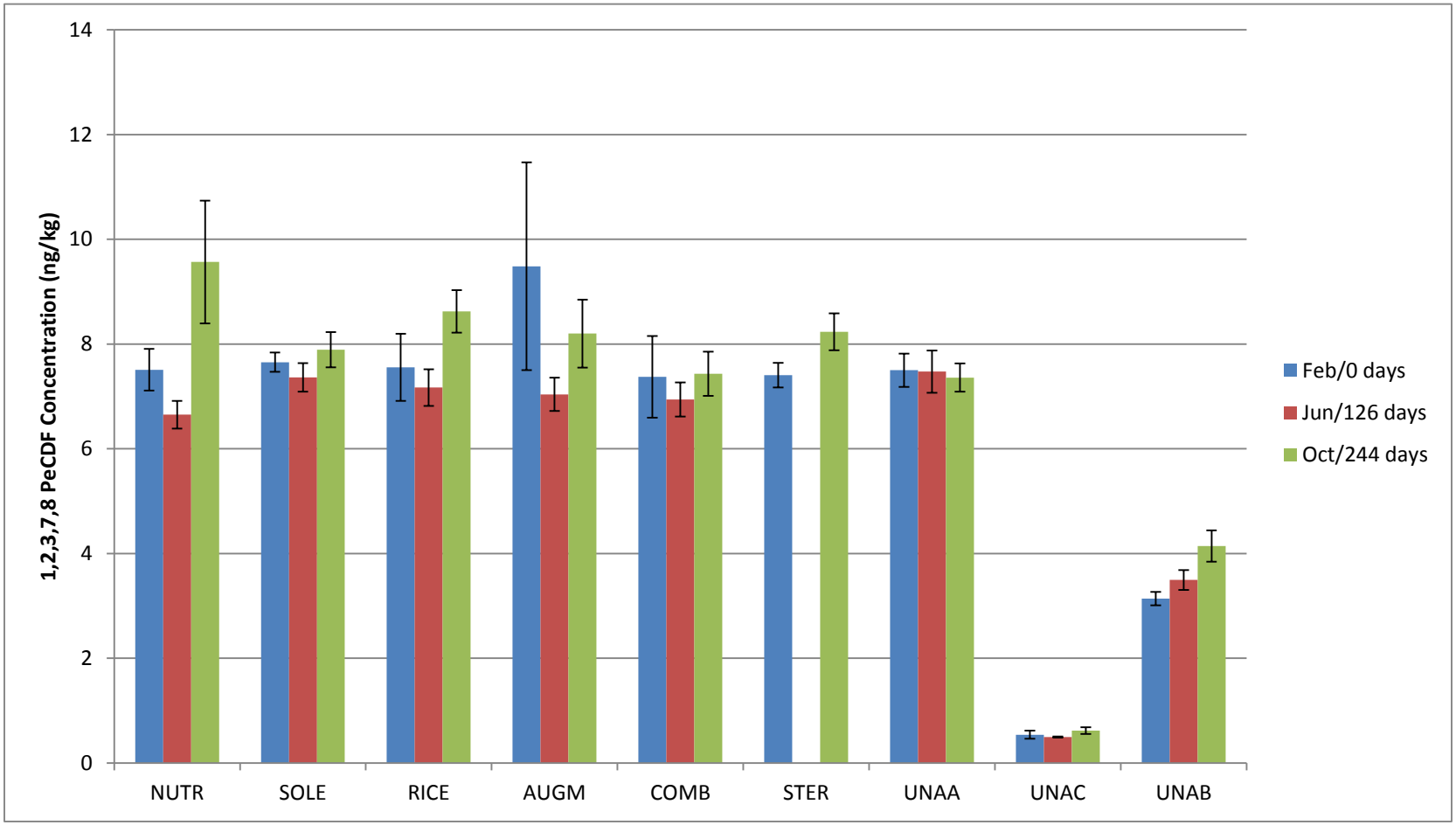

Figure E-10: 1,2,3,7,8 PeCDF concentrations during microcosm incubation

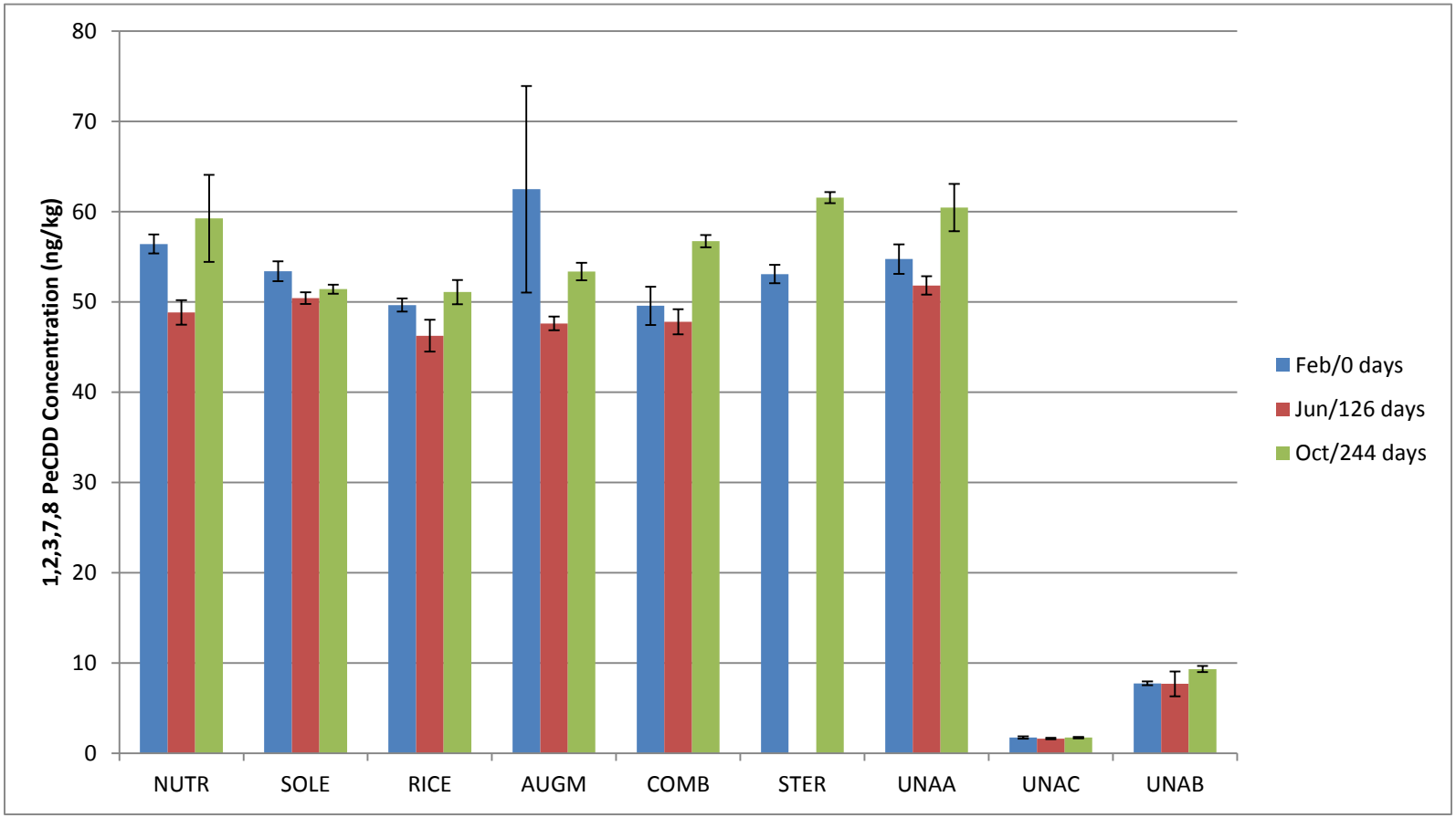

Figure E-11: 1,2,3,7,8 PeCDD concentrations during microcosm incubation

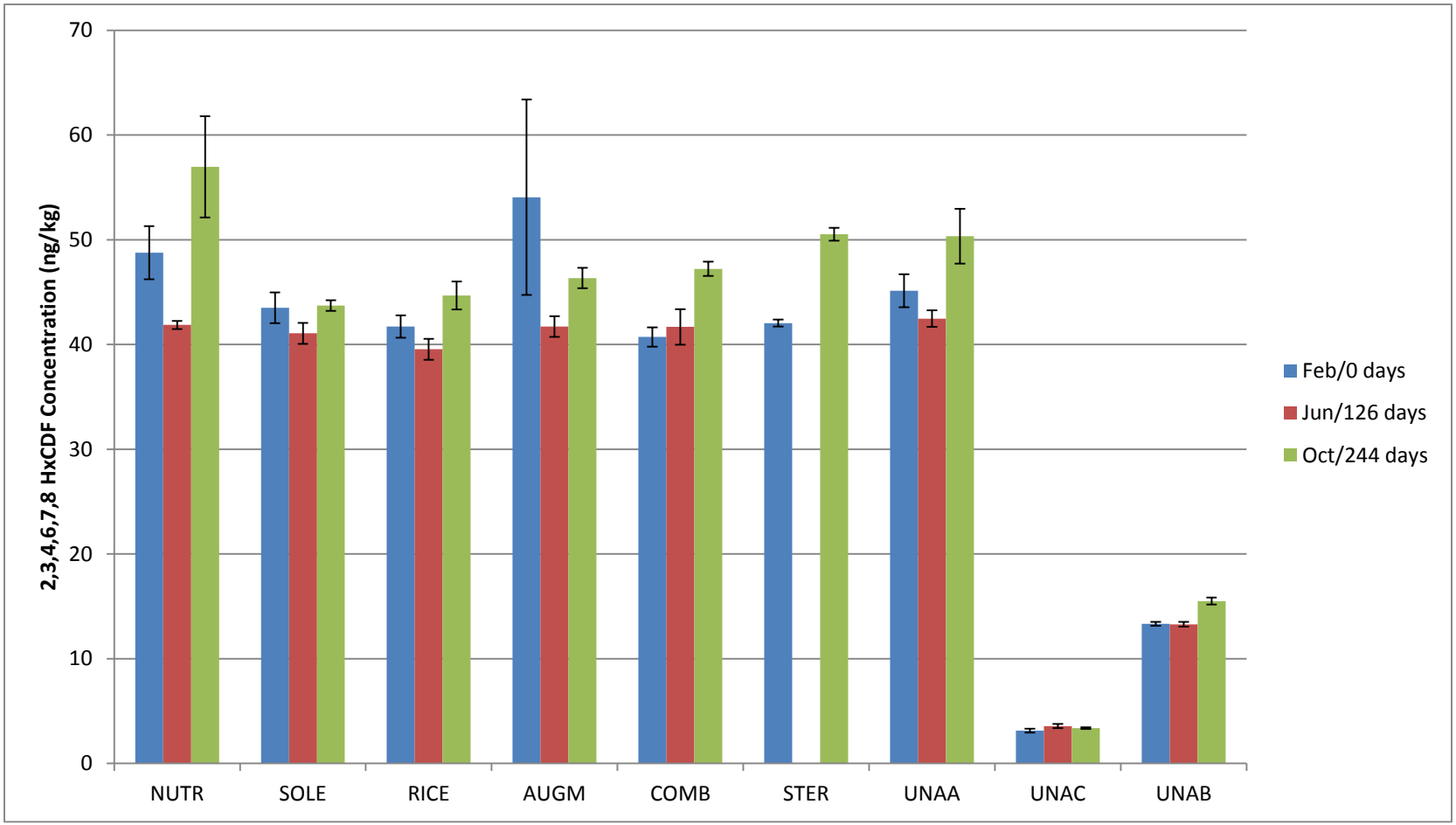

Figure E-12: 2,3,4,6,7,8 HxCDF concentrations during microcosm incubation

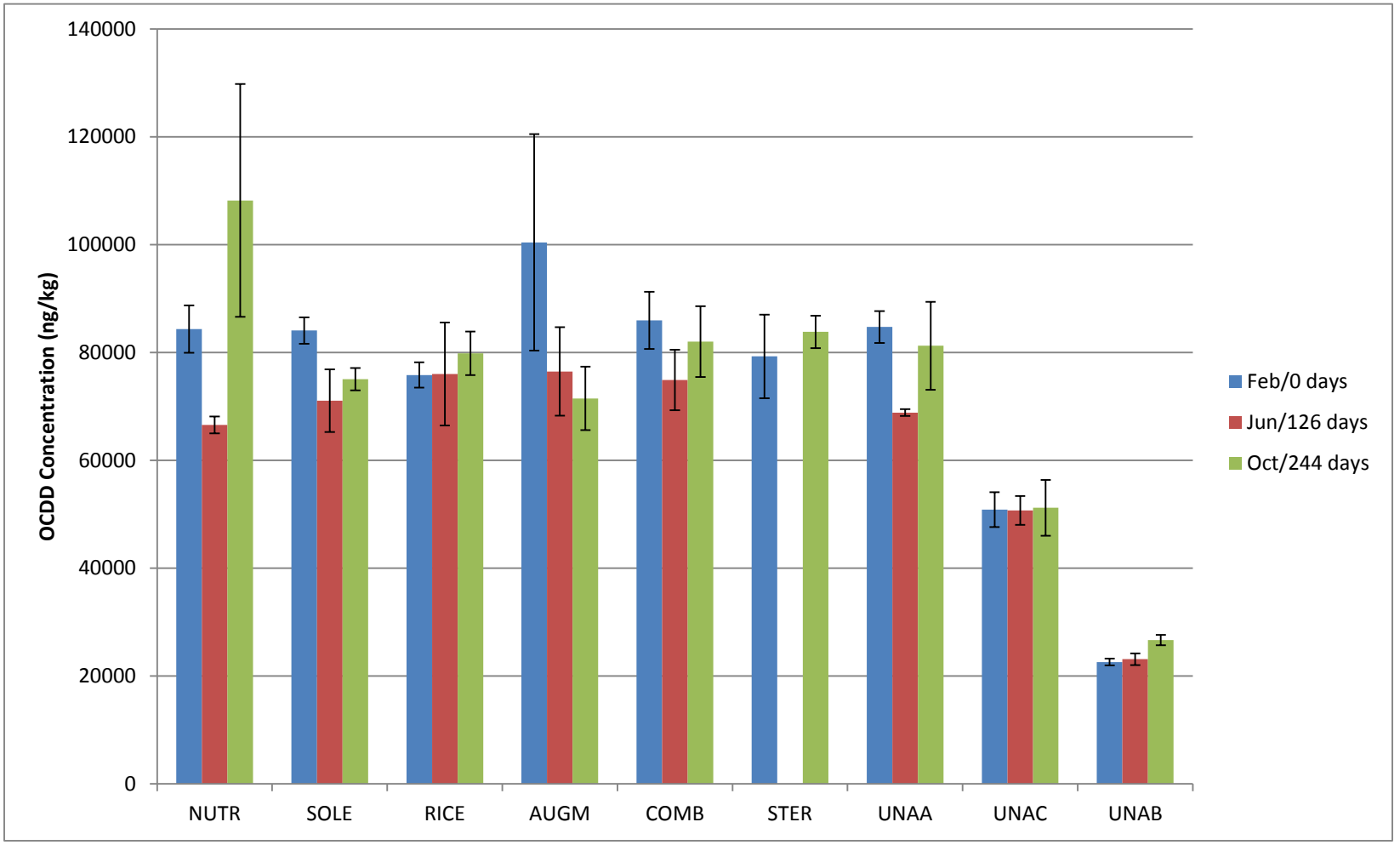

Figure E-13: OCDD concentrations during microcosm incubation

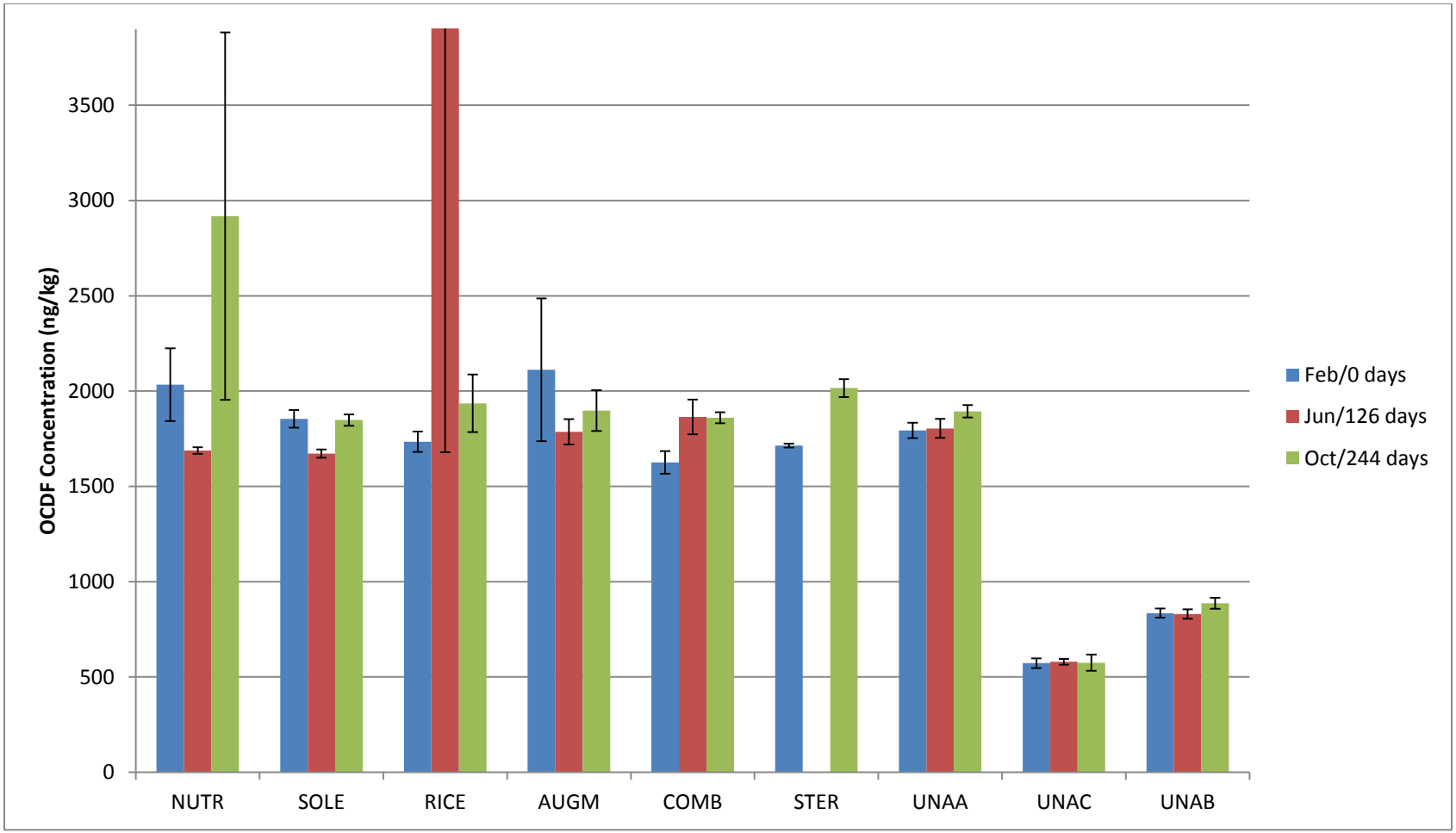

Figure E-14: Truncated OCDF concentrations during microcosm incubation

Appendix F: Microcosm Results for Individual PAH Compound Concentrations

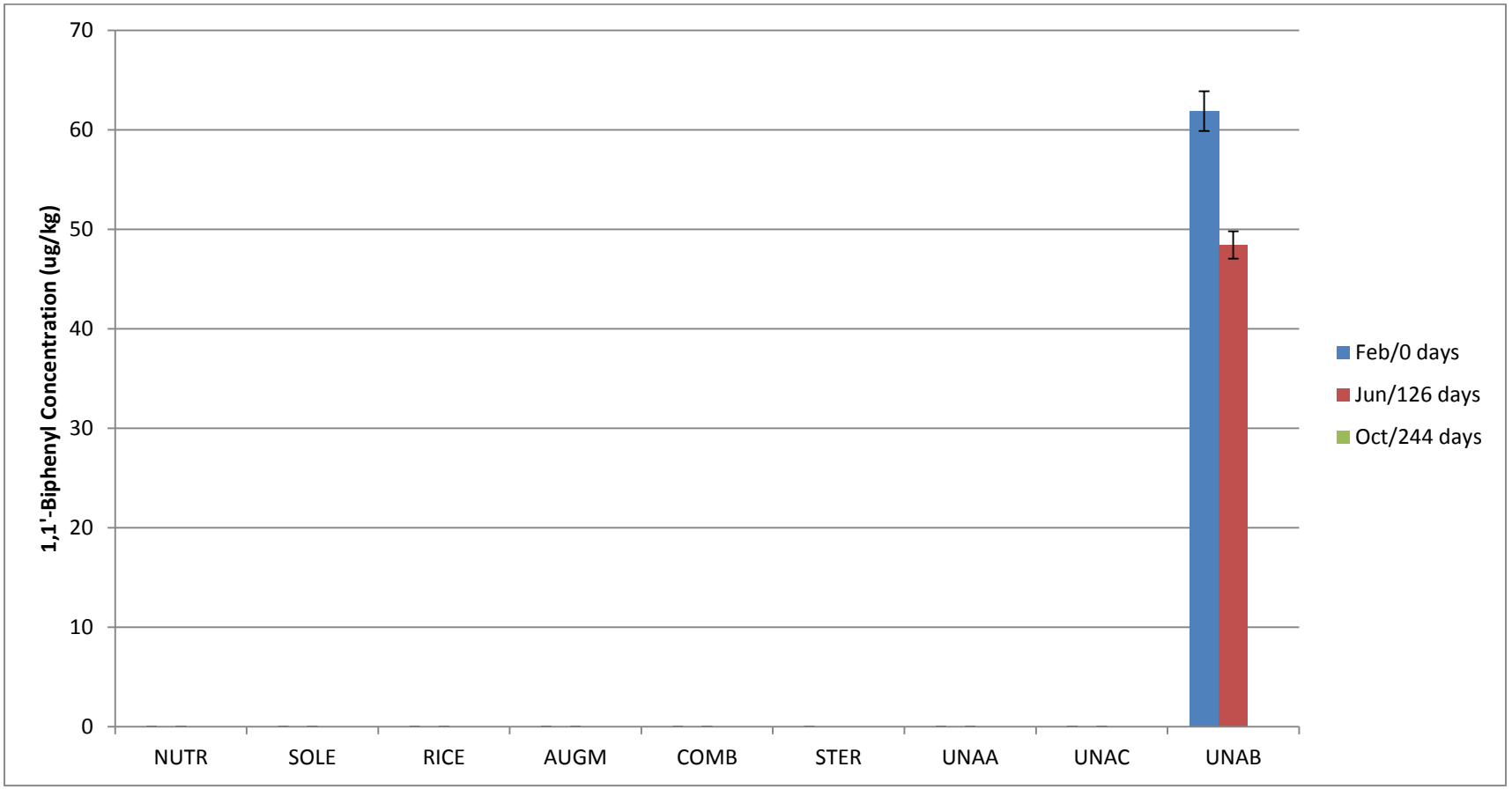

Figure F-1: 1,1'-biphenyl concentrations during microcosm incubation

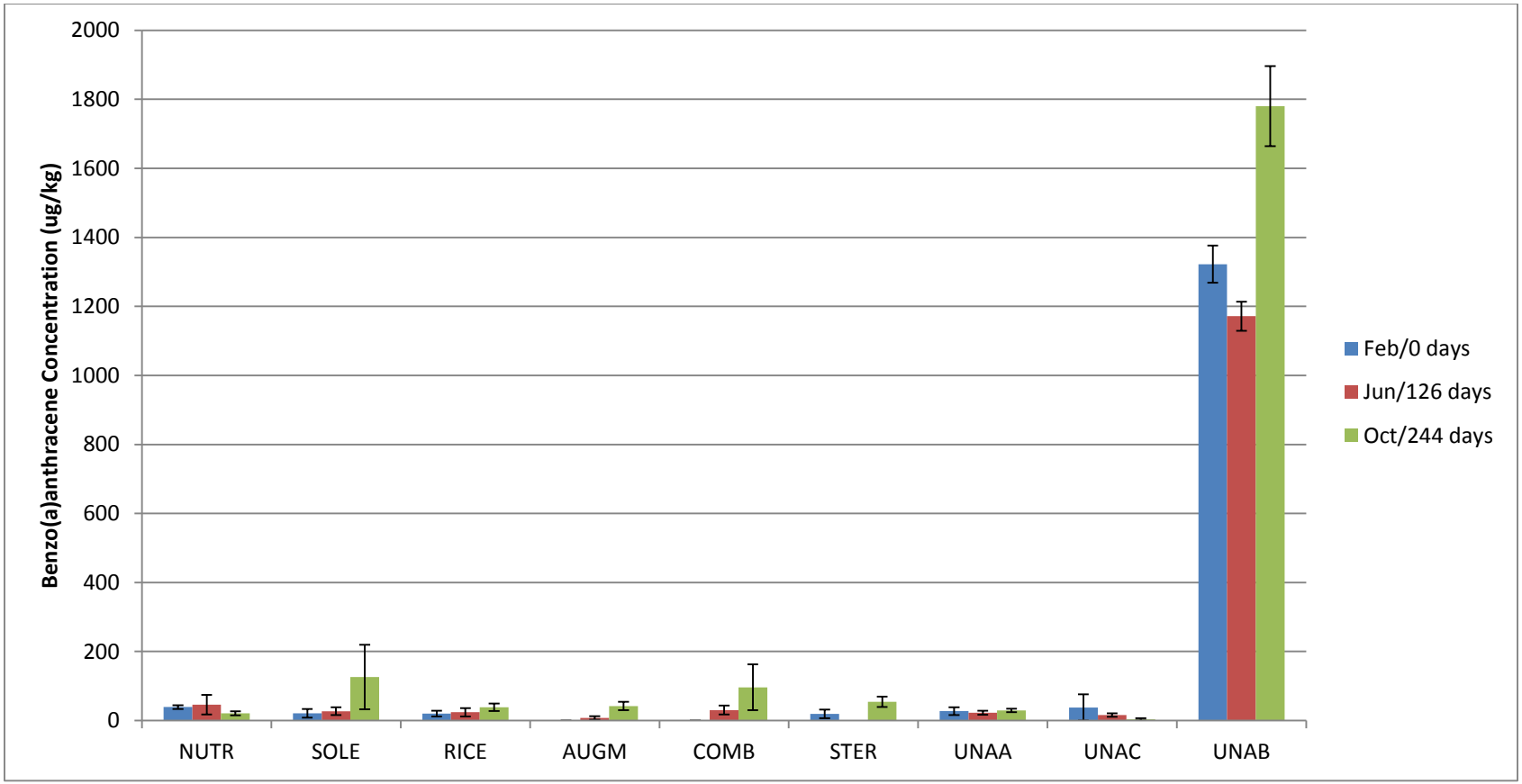

Figure F-2: Benzo(a)anthracene concentrations during microcosm incubation (all soils)

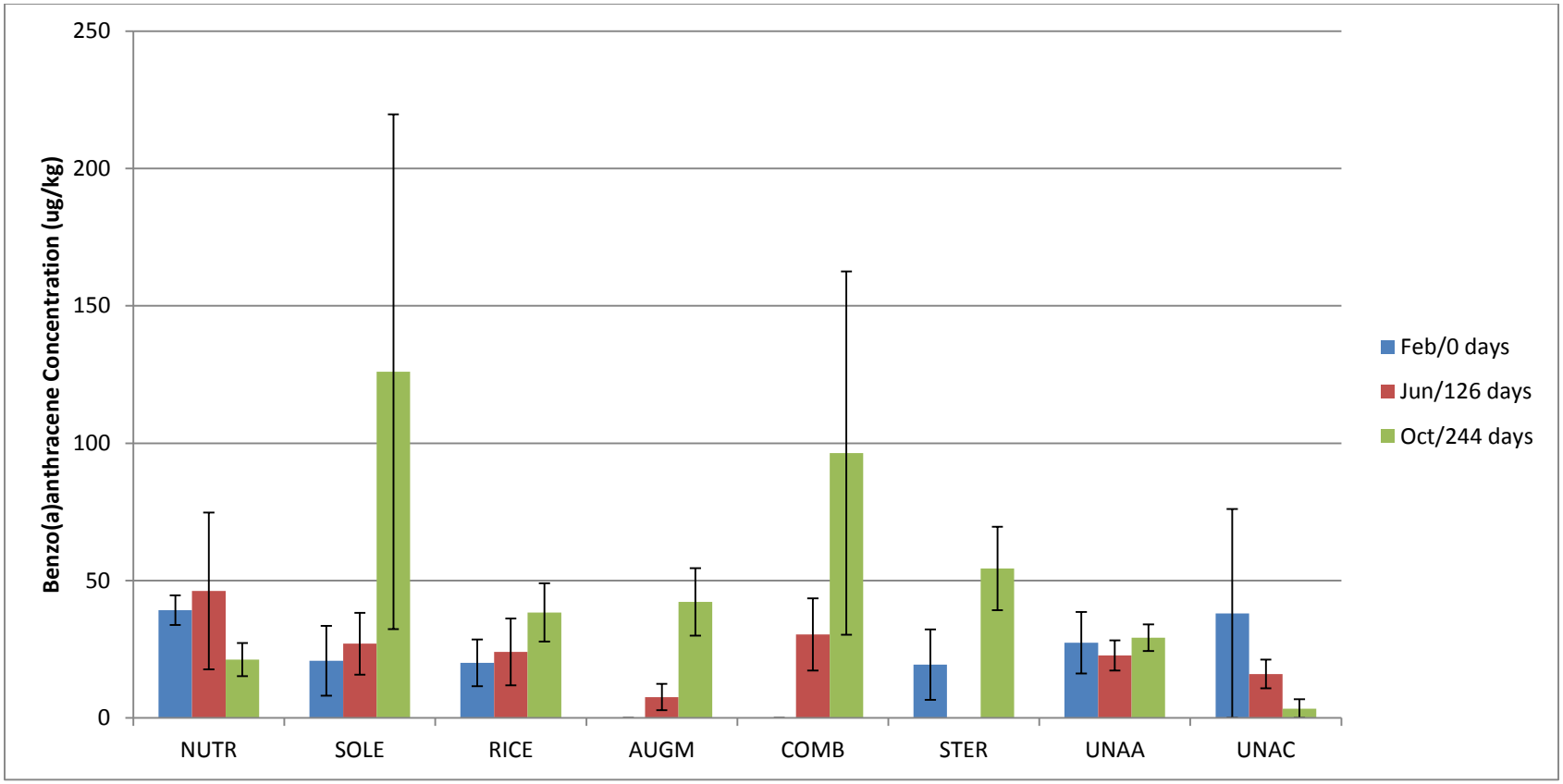

Figure F-3: Benzo(a)anthracene during incubation (Soils A and C only)

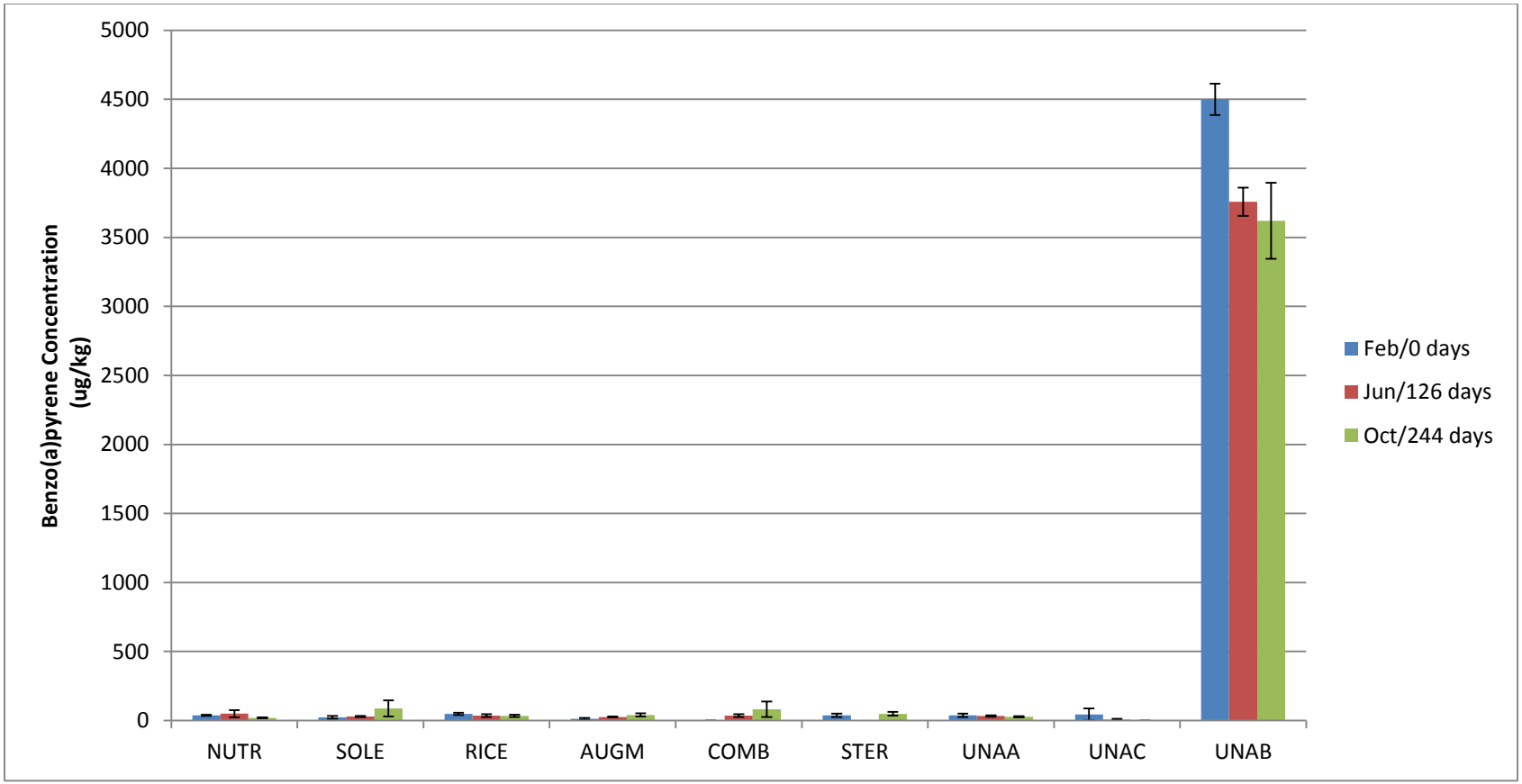

Figure F-4: Benzo(a)pyrene concentrations during microcosm incubation (all soils)

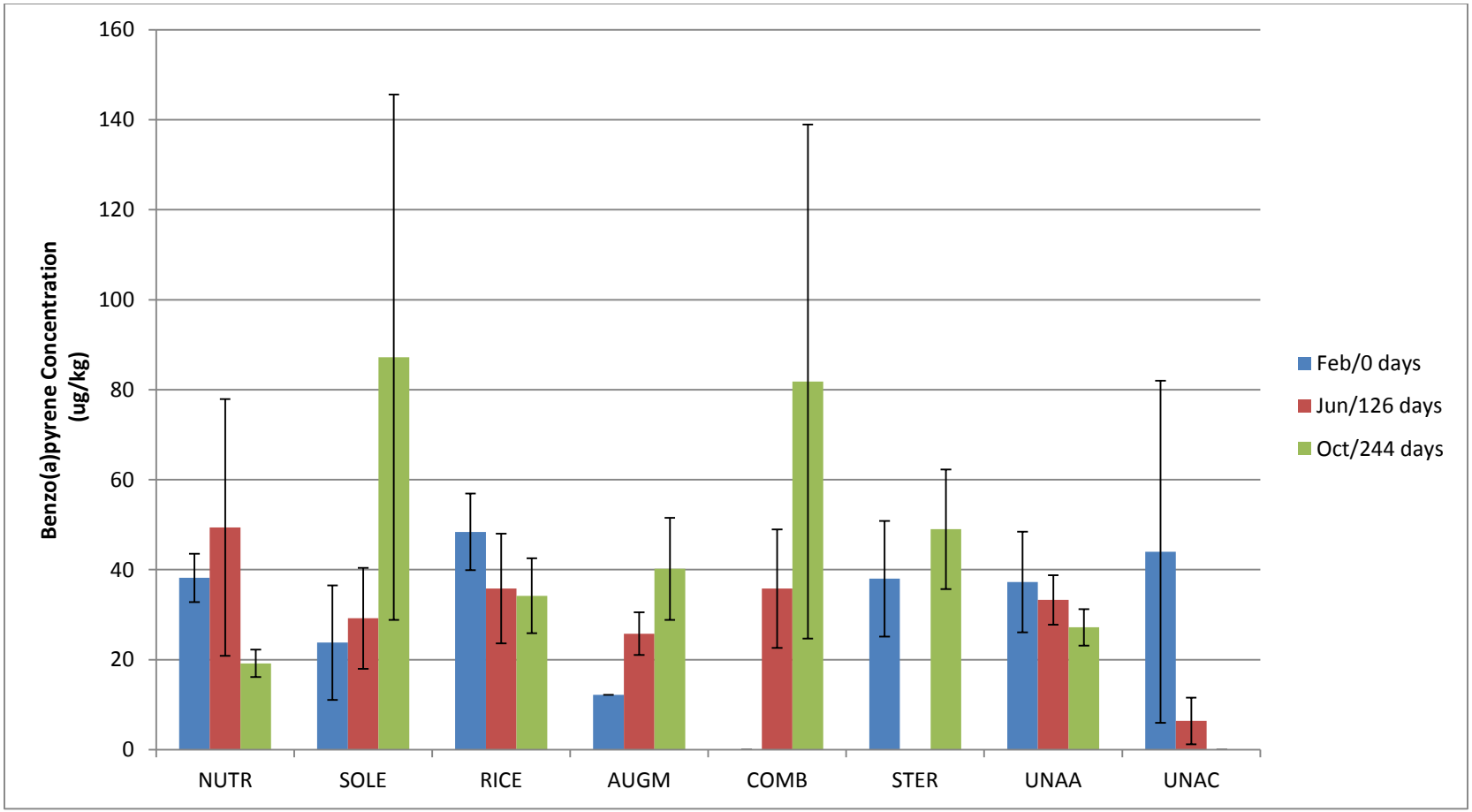

Figure F-5: Benzo(a)pyrene concentrations during microcosm incubation (Soils A and C)

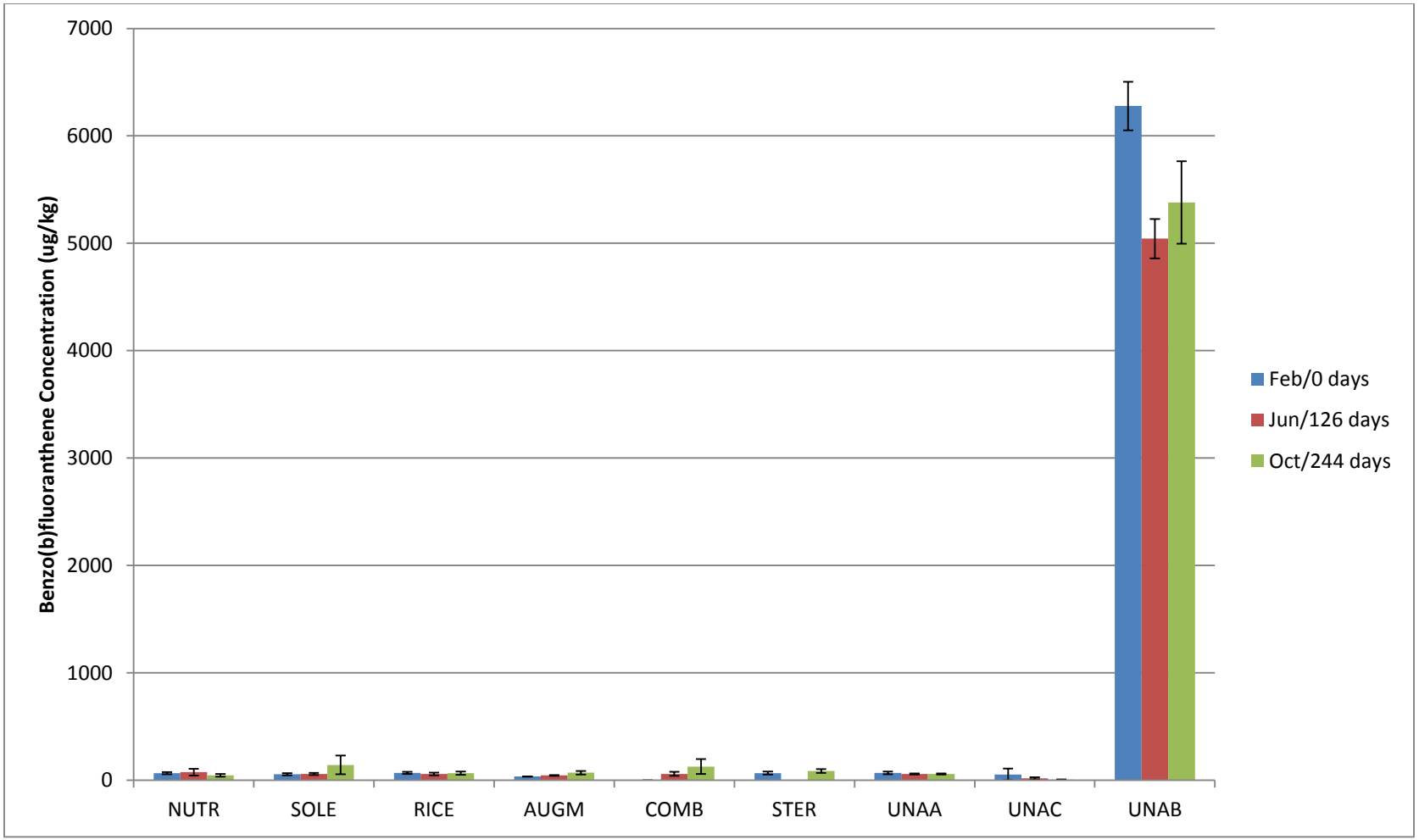

Figure F-6: Benzo(b)fluoranthene concentrations during microcosm incubation (all soils)

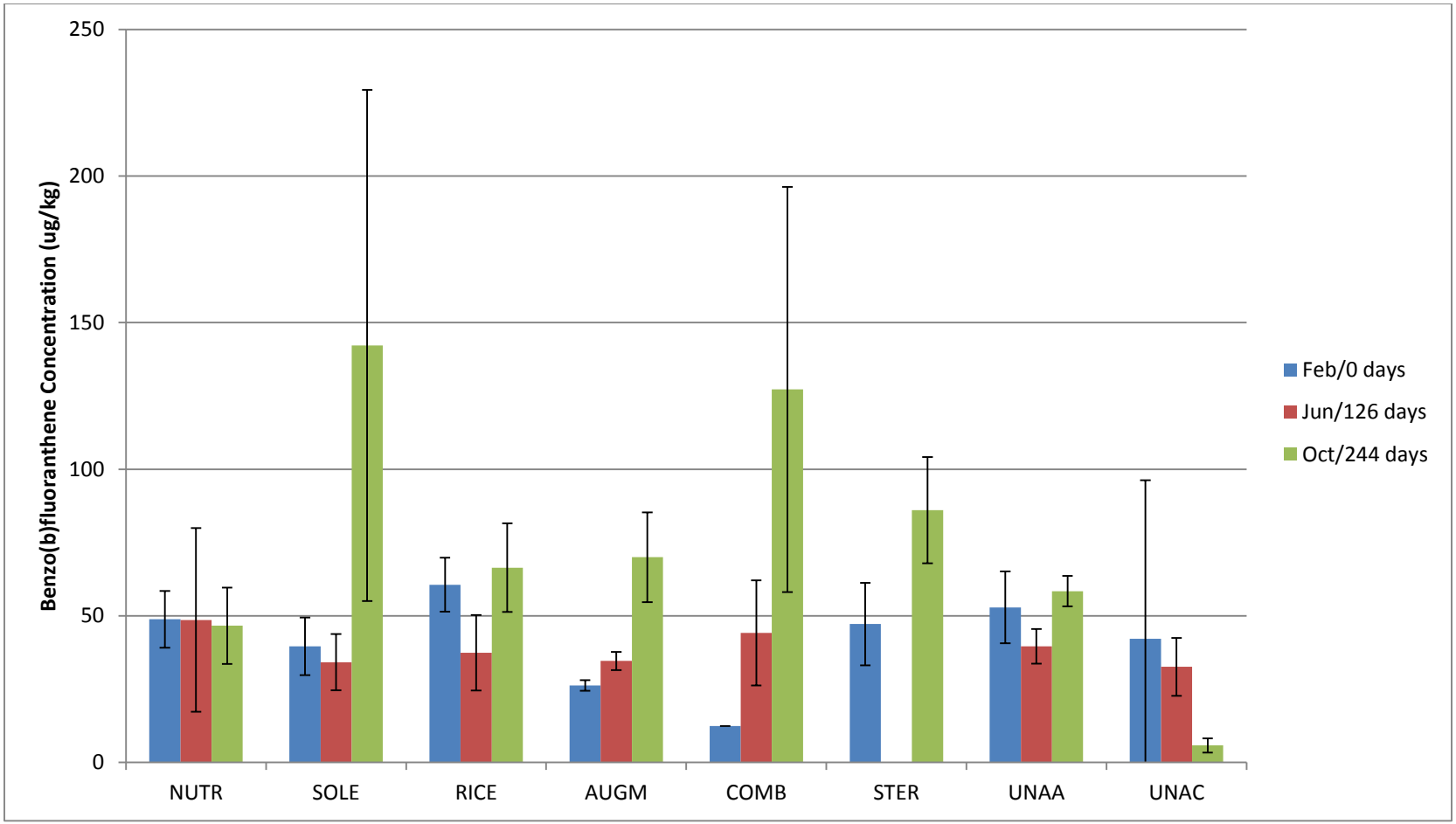

Figure F-7: Benzo(b)fluoranthene concentrations during microcosm incubation (Soils A and C)

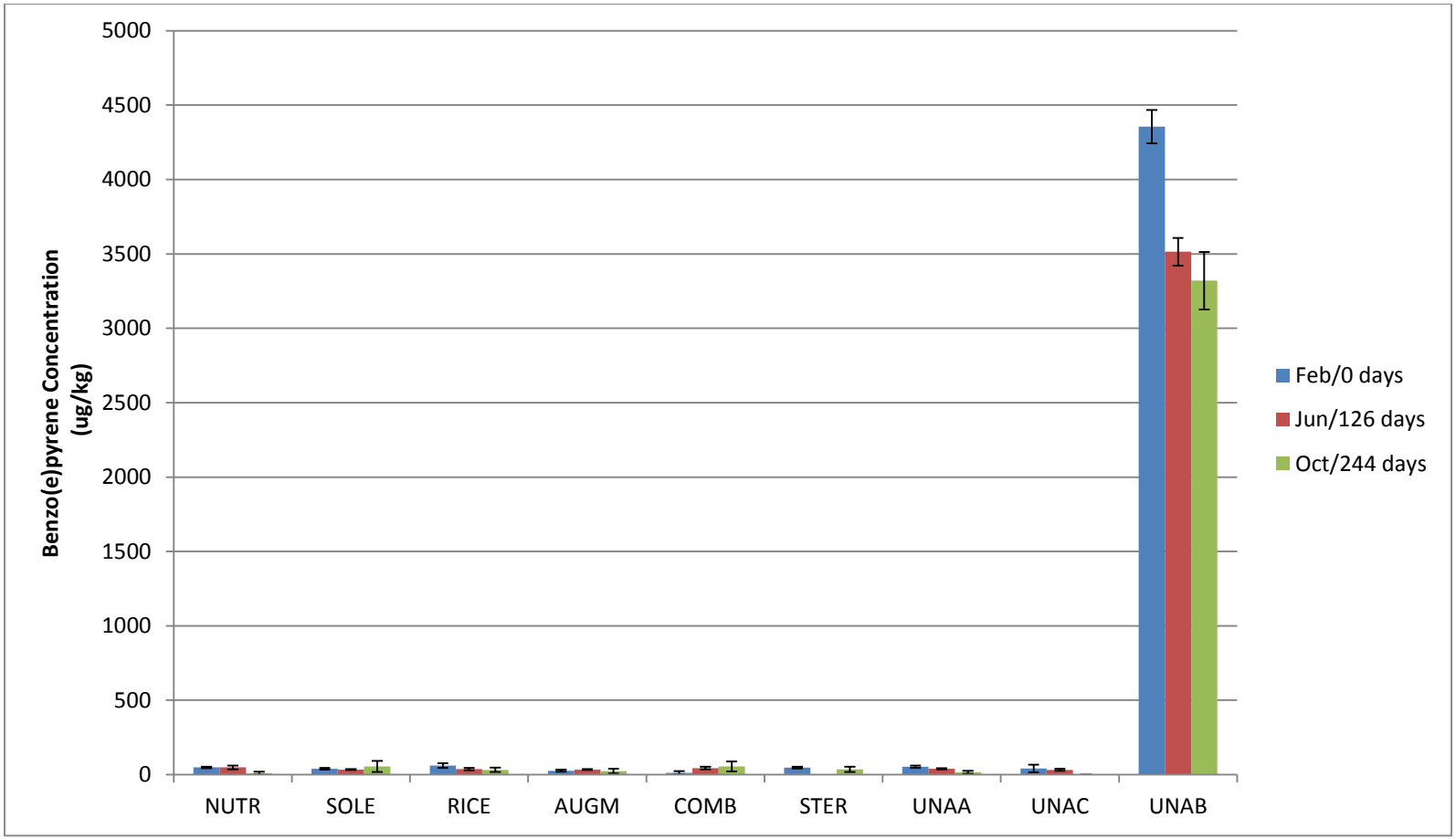

Figure F-8: Benzo(e)pyrene concentrations during microcosm incubation (all soils)

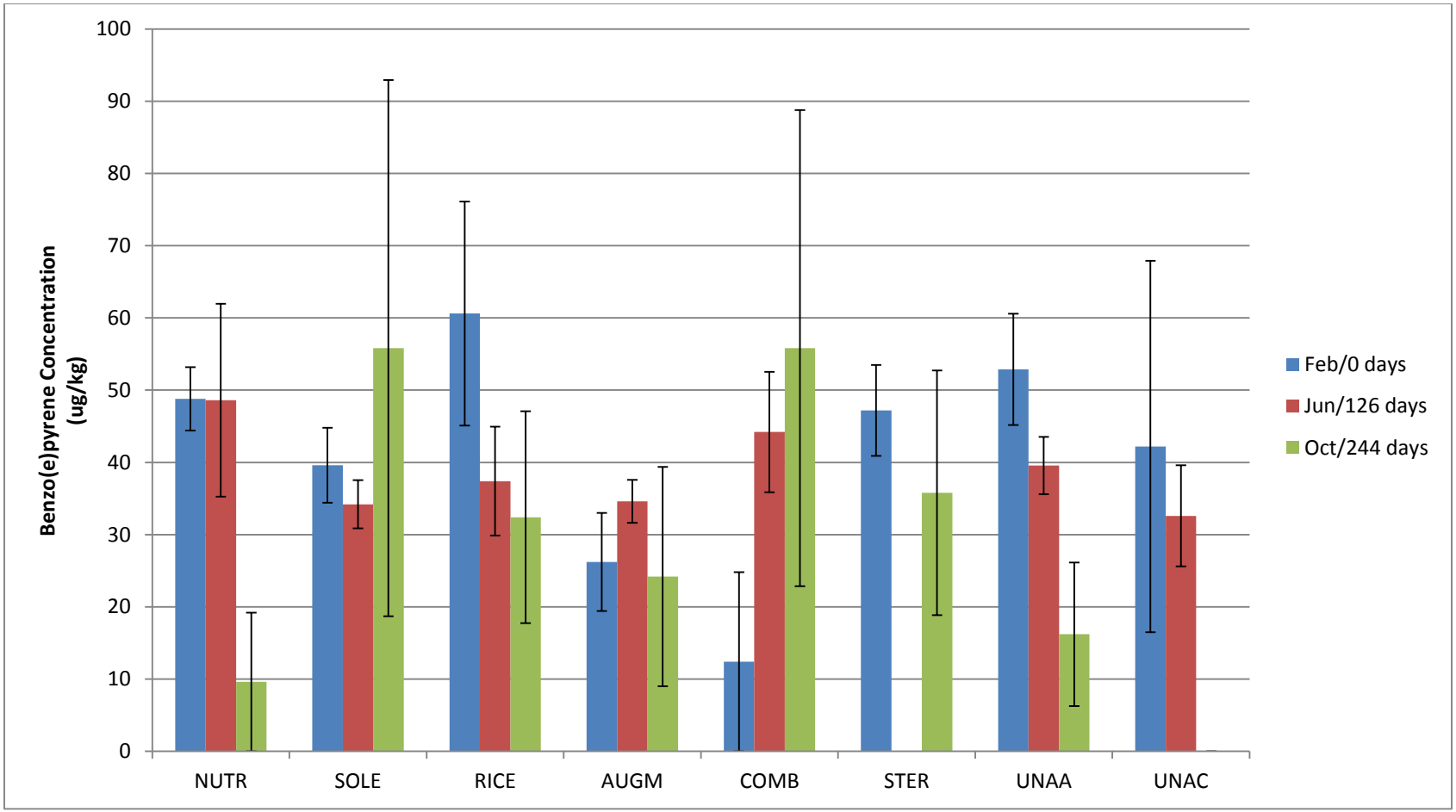

Figure F-9: Benzo(e)pyrene concentrations during microcosm incubation (Soils A and C)

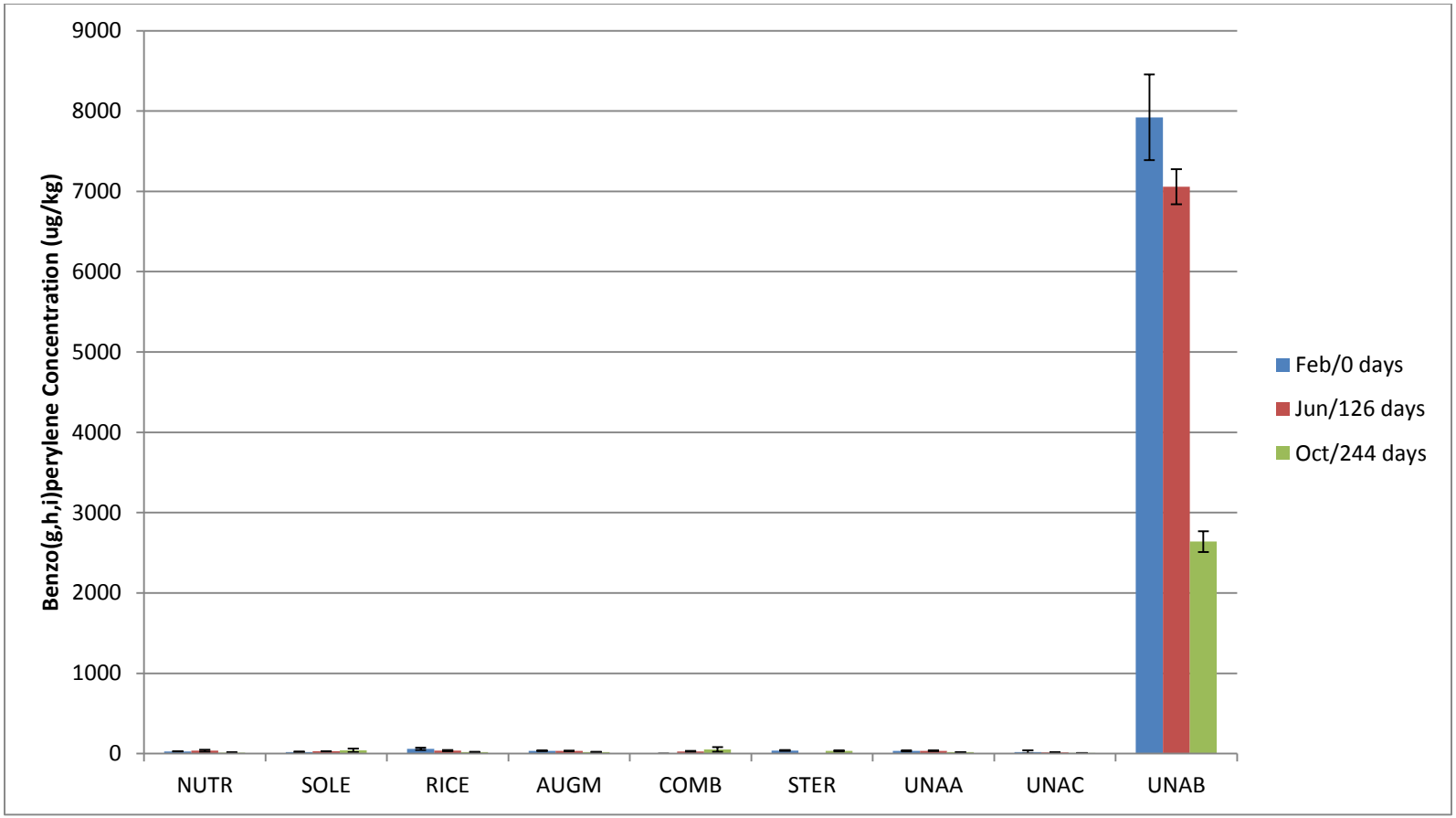

Figure F-10: Benzo(g,h,i)perylene concentrations during microcosm incubation (all soils)

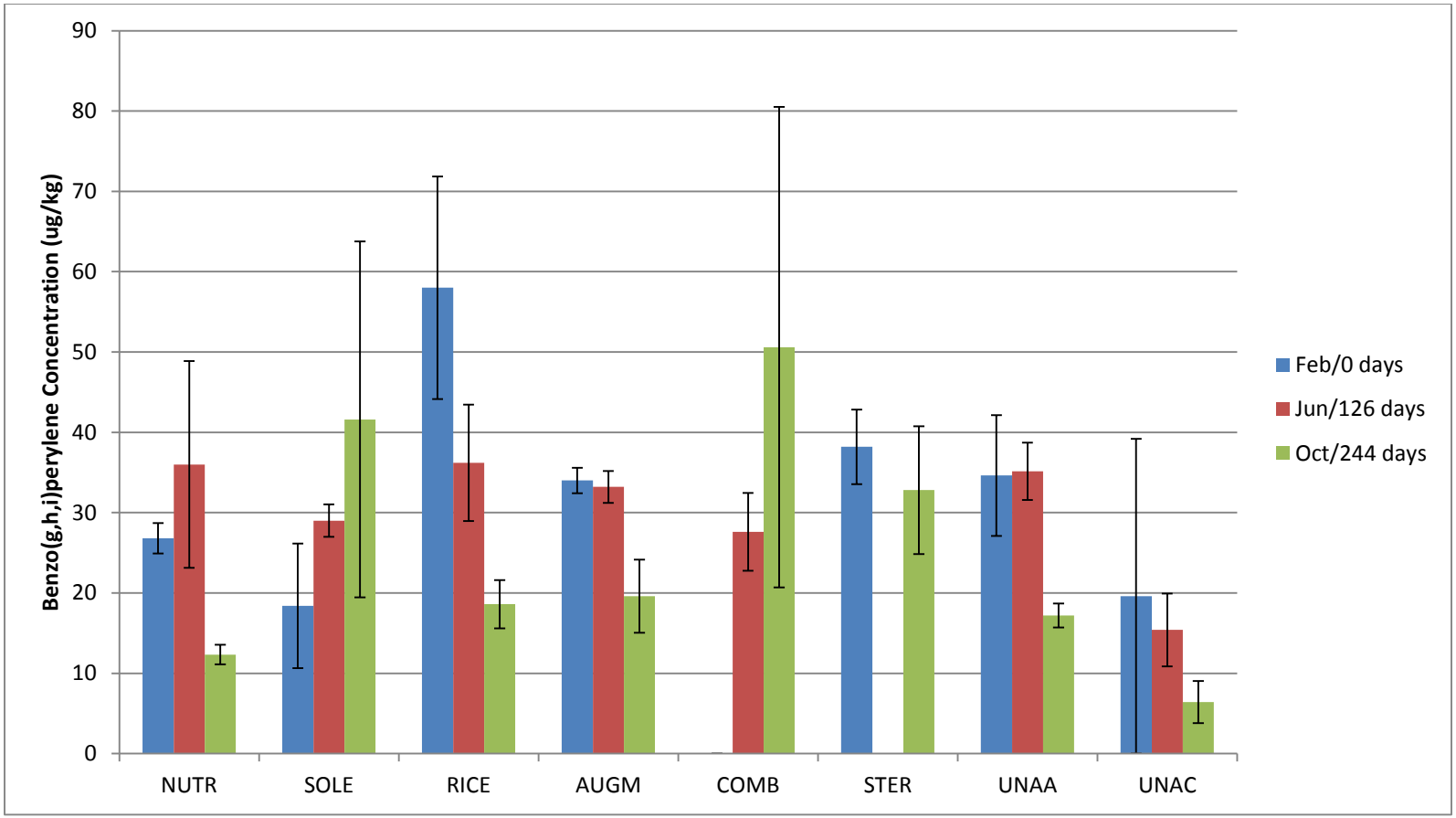

Figure F-11: Benzo(g,h,i)perylene concentrations during microcosm incubation (Soils A and C)

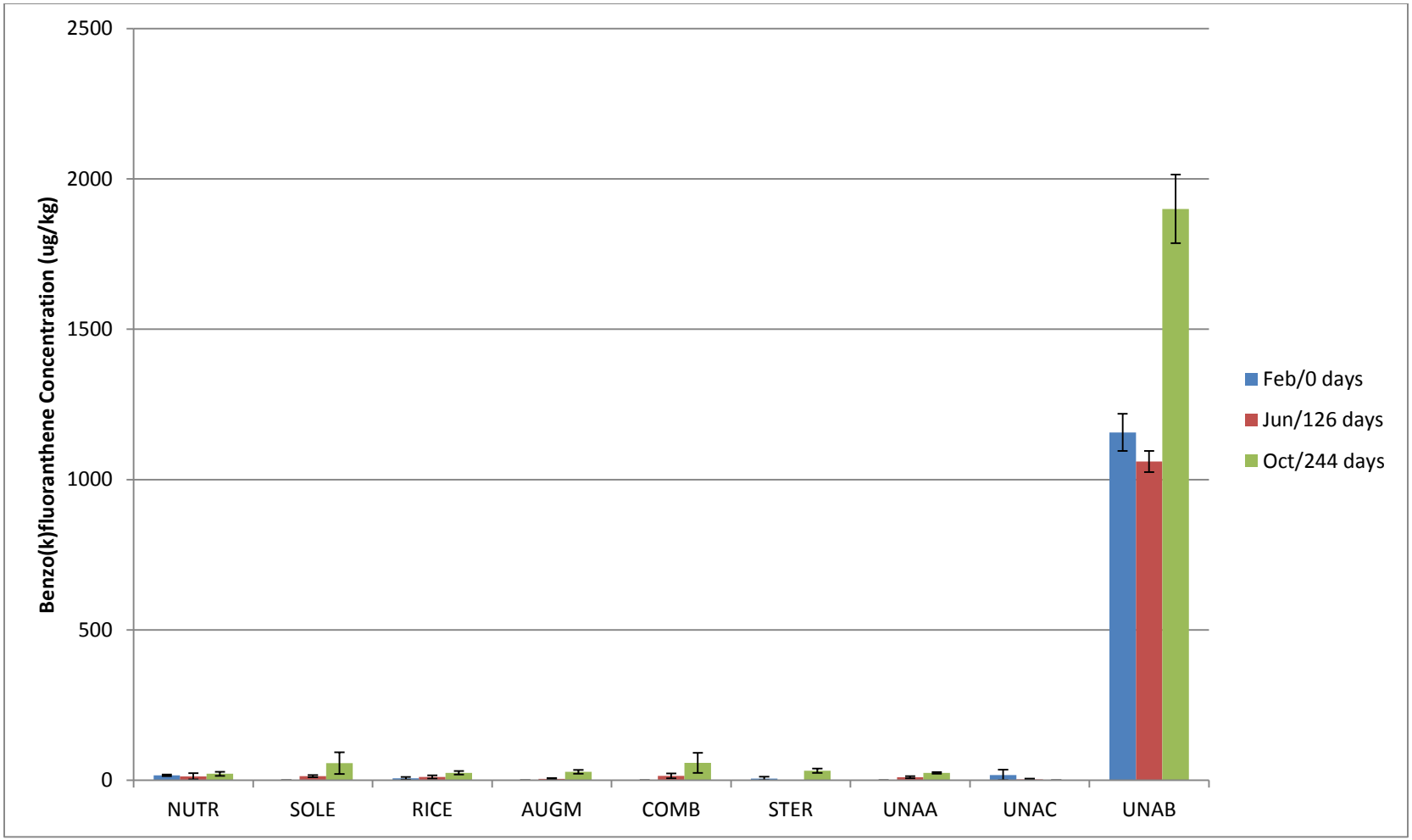

Figure F-12: Benzo(k)fluoranthene concentrations during microcosm incubation (all soils)

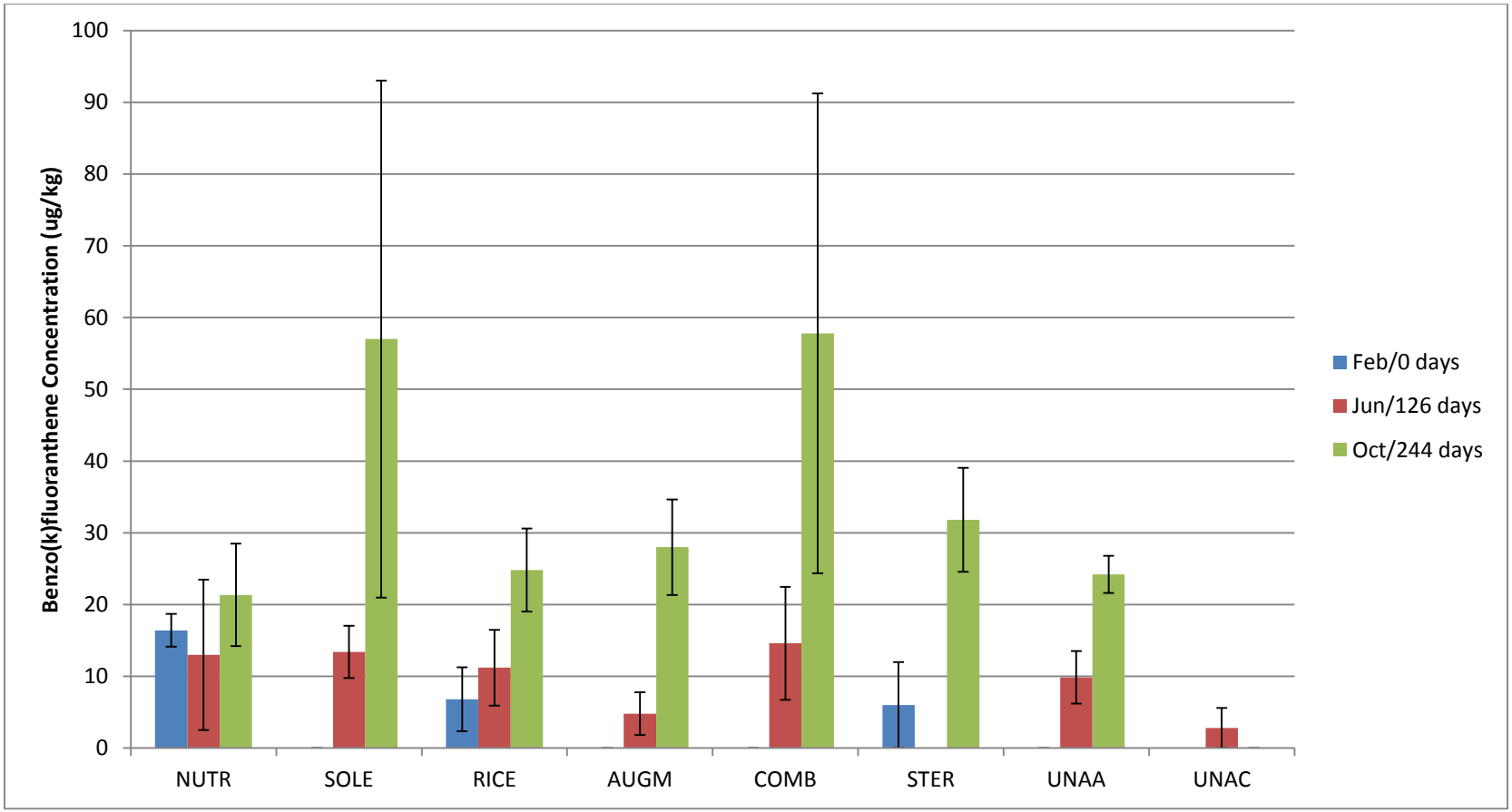

Figure F-13: Benzo(k)fluoranthene concentrations during microcosm incubation (Soils A and C)

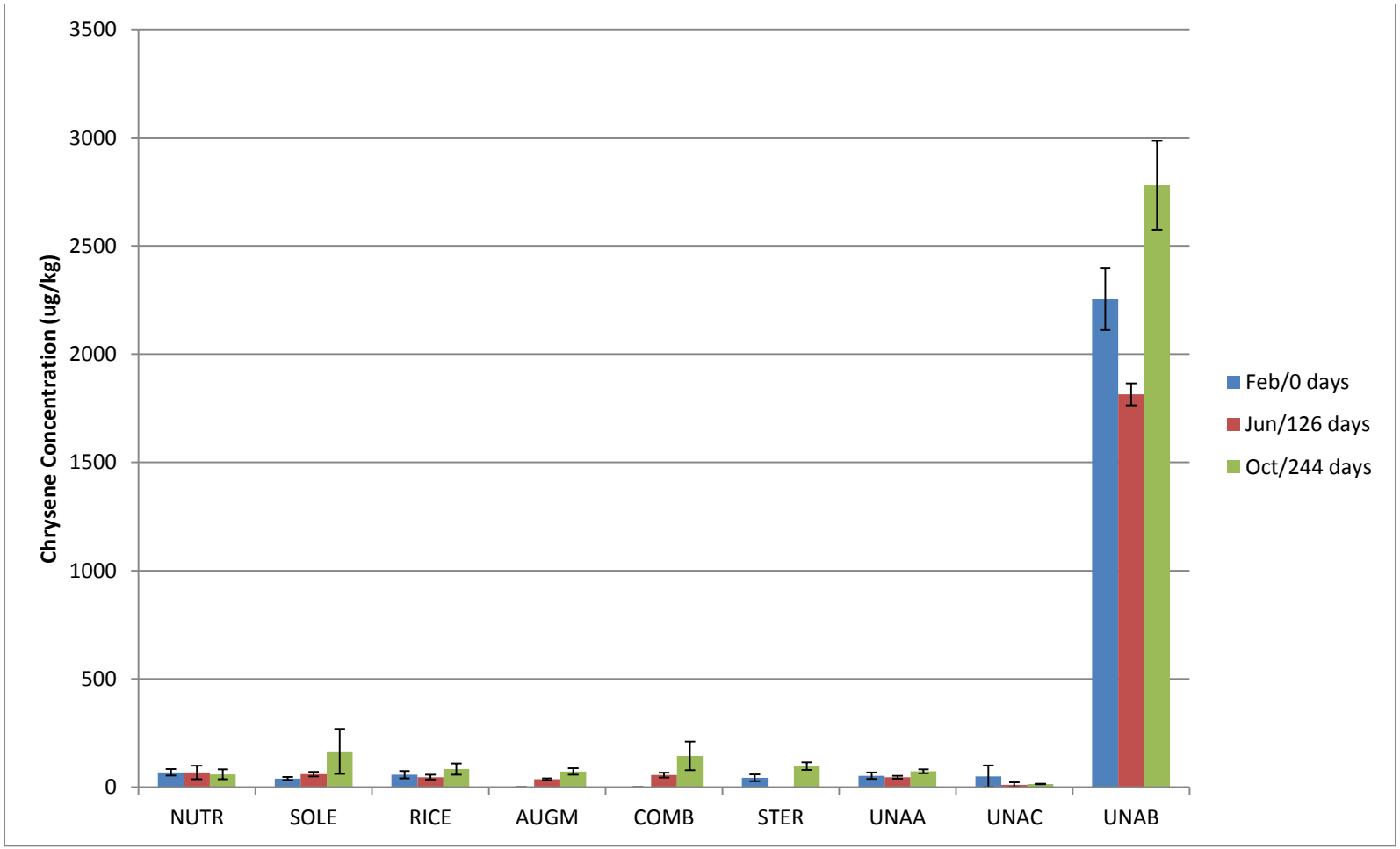

Figure F-14: Chrysene concentrations during microcosm incubation (all soils)

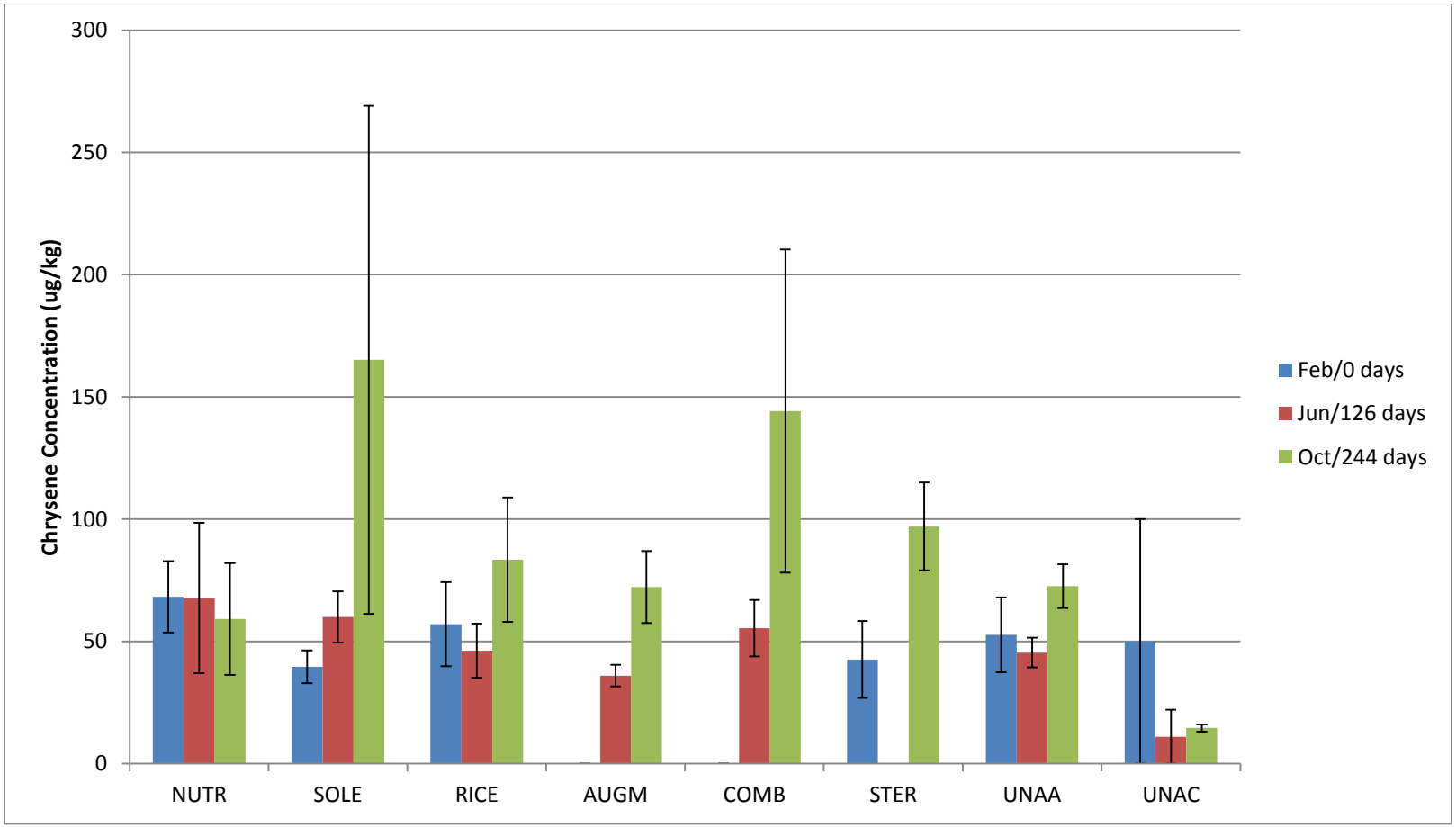

Figure F-15: Chrysene concentrations during microcosm incubation (Soils A and C)

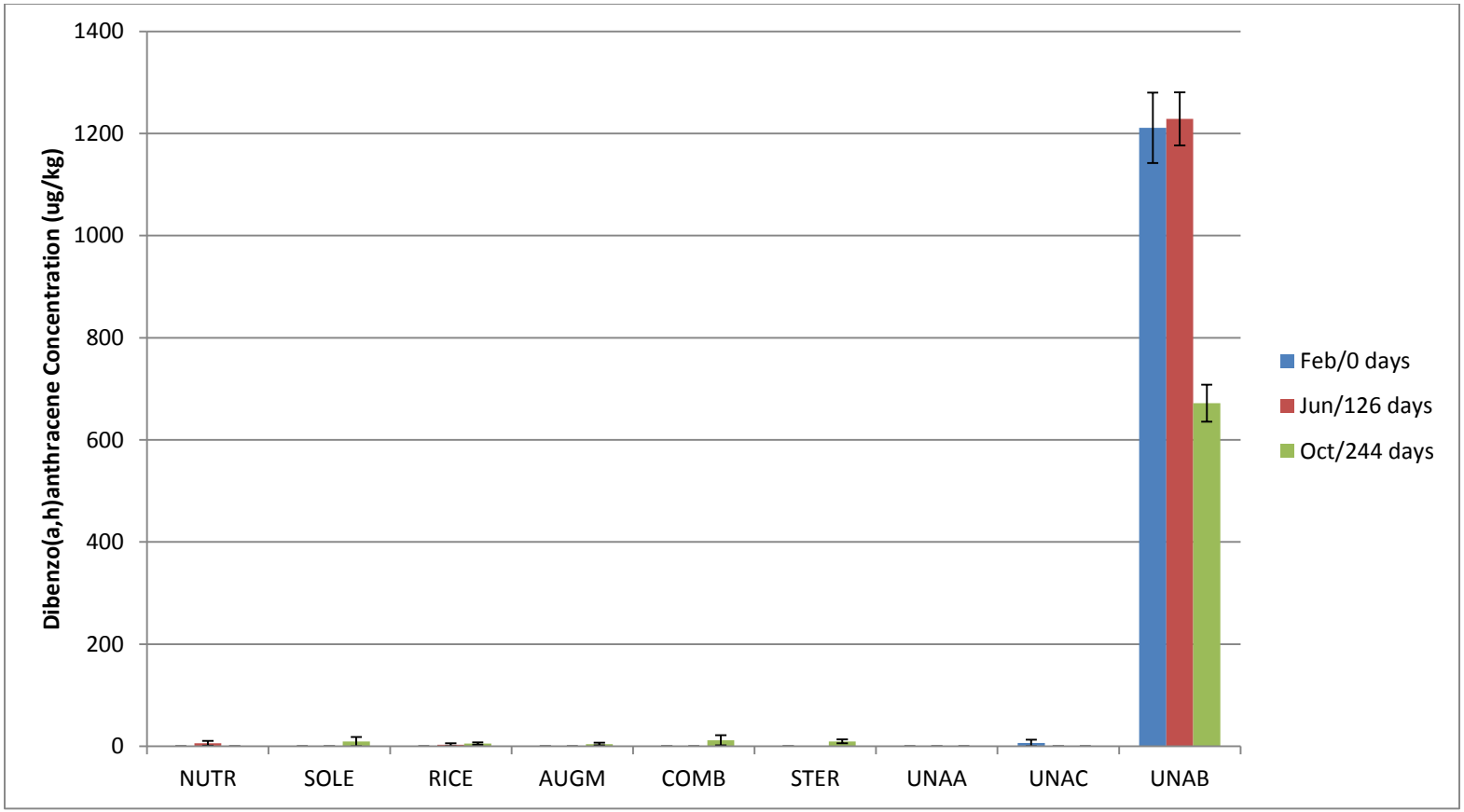

Figure F-16: Dibenzo(a,h)anthracene concentrations during microcosm incubation (all soils)

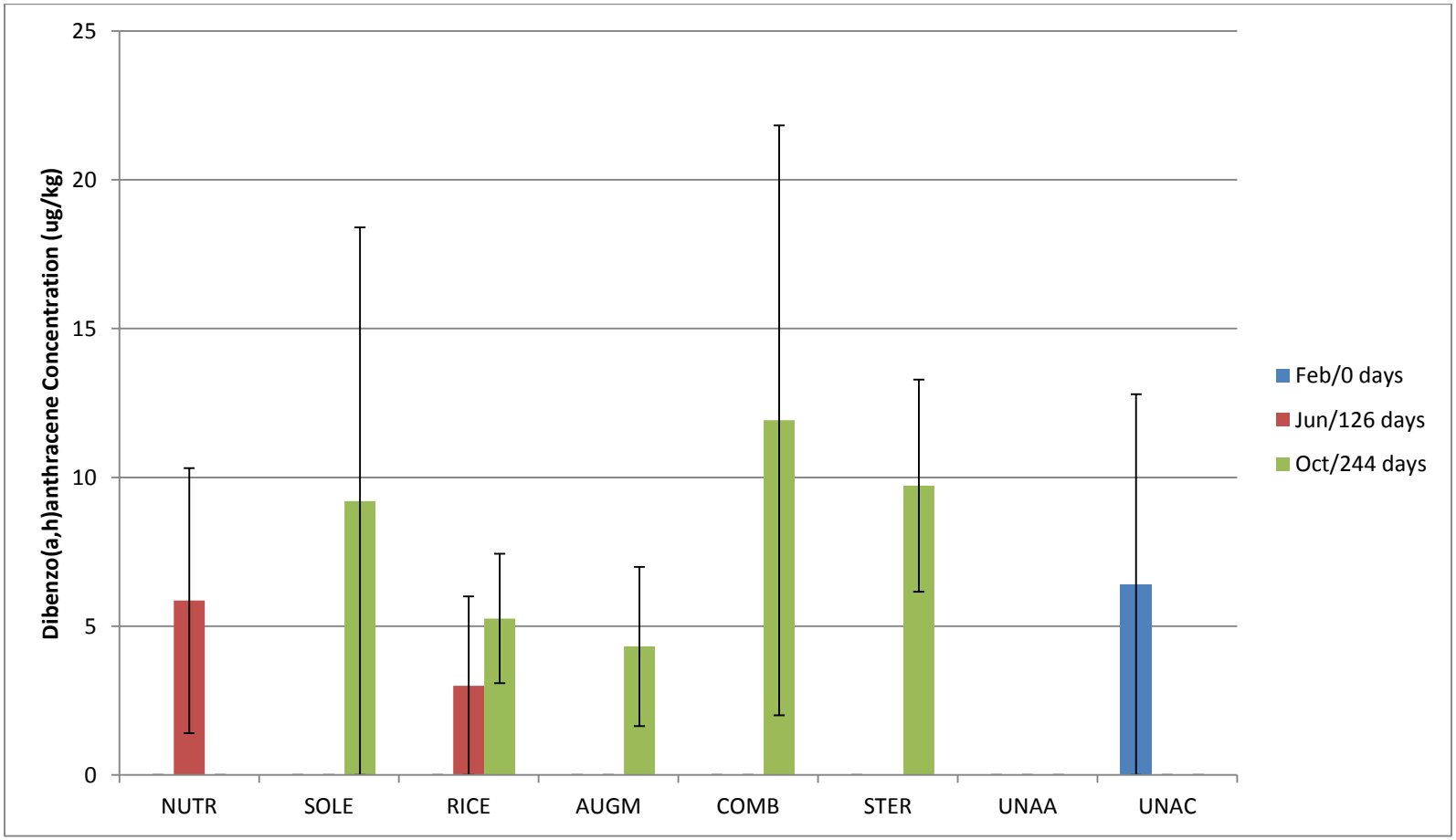

Figure F-17: Dibenzo(a,h)anthracene concentrations during microcosm incubation (Soils A and C)

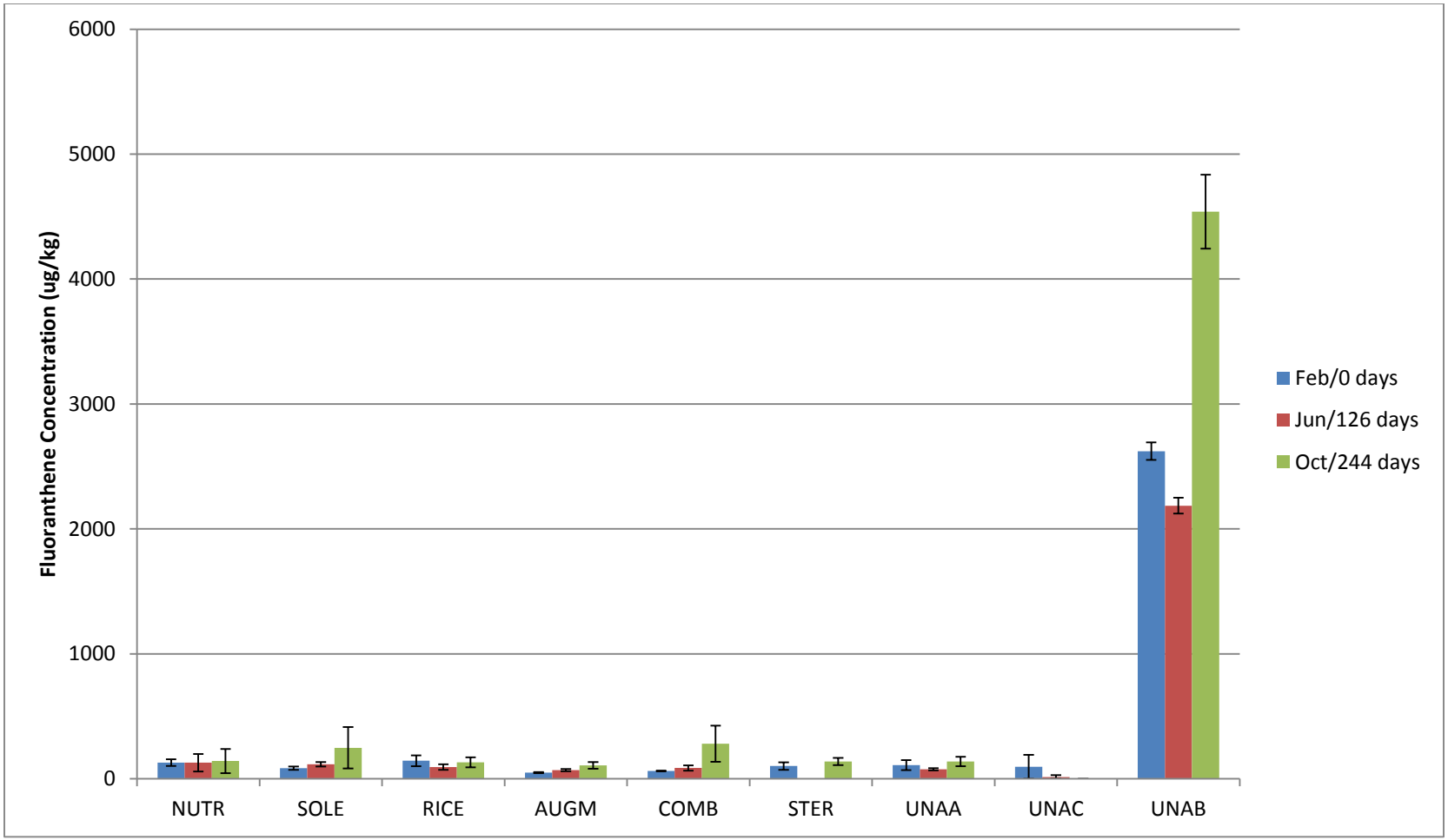

Figure F-18: Fluoranthene concentrations during microcosm incubation (all soils)

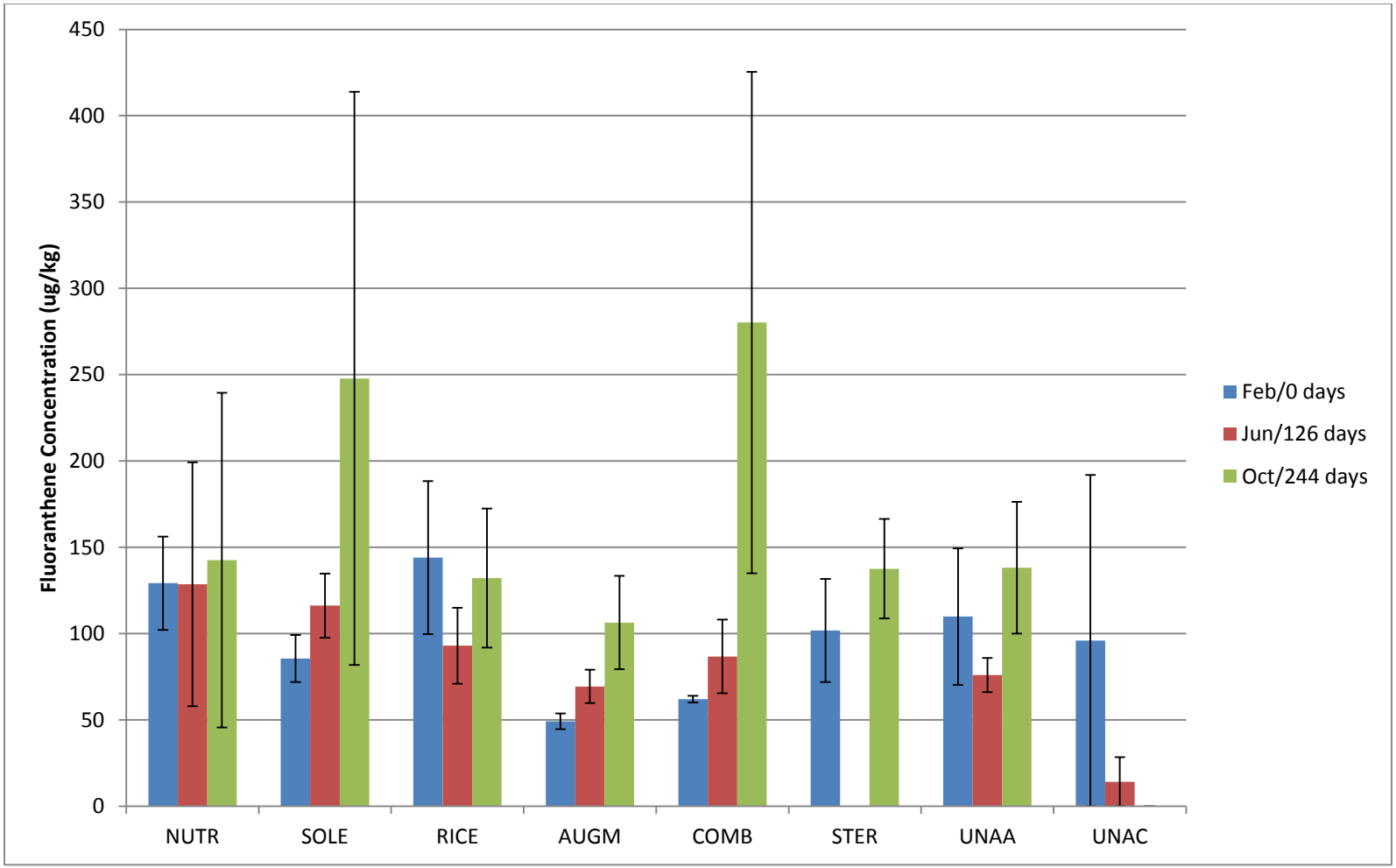

Figure F-19: Flouranthene concentrations during microcosm incubation (Soils A and C)

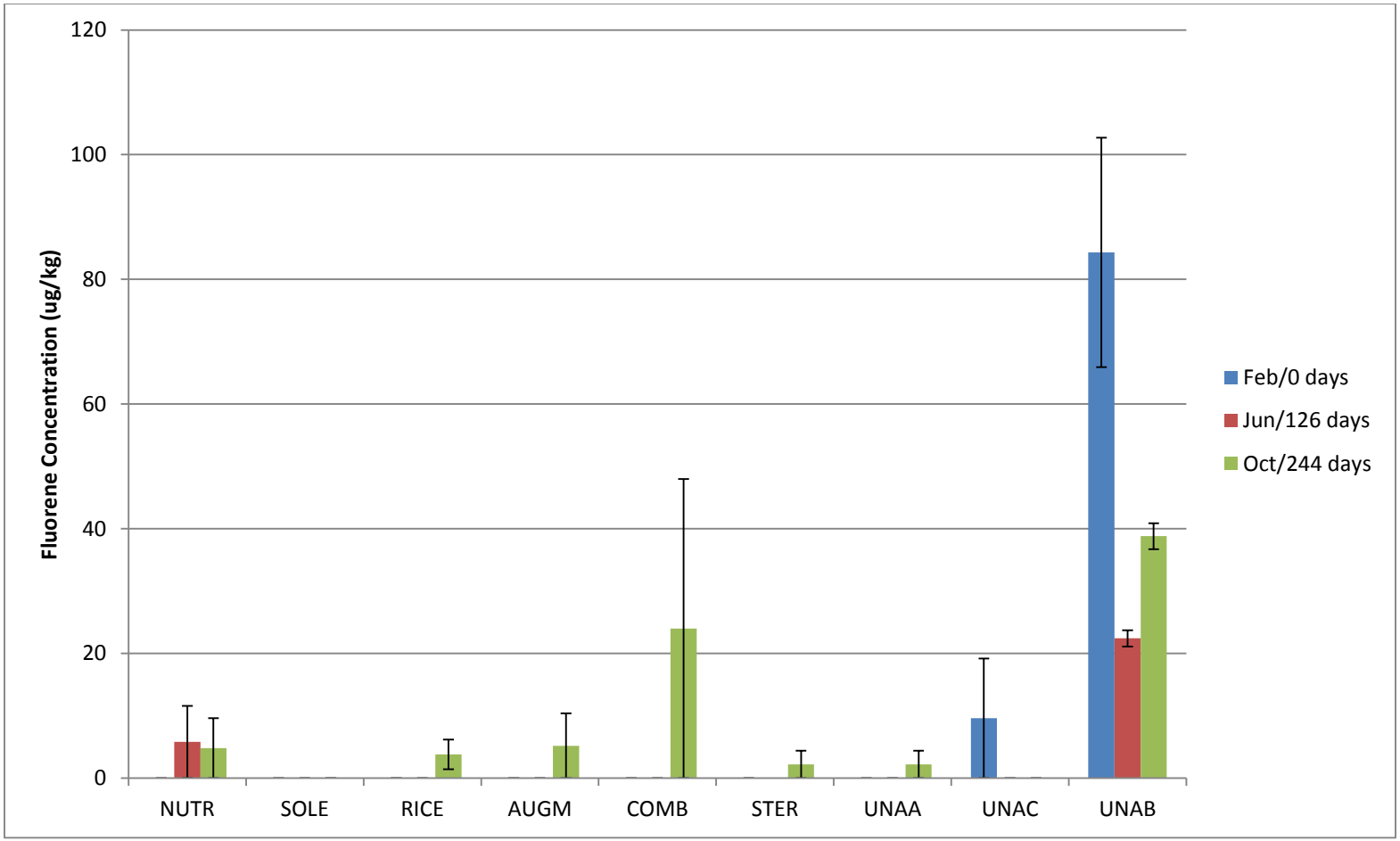

Figure F-20: Fluorene concentrations during microcosm incubation (all soils)

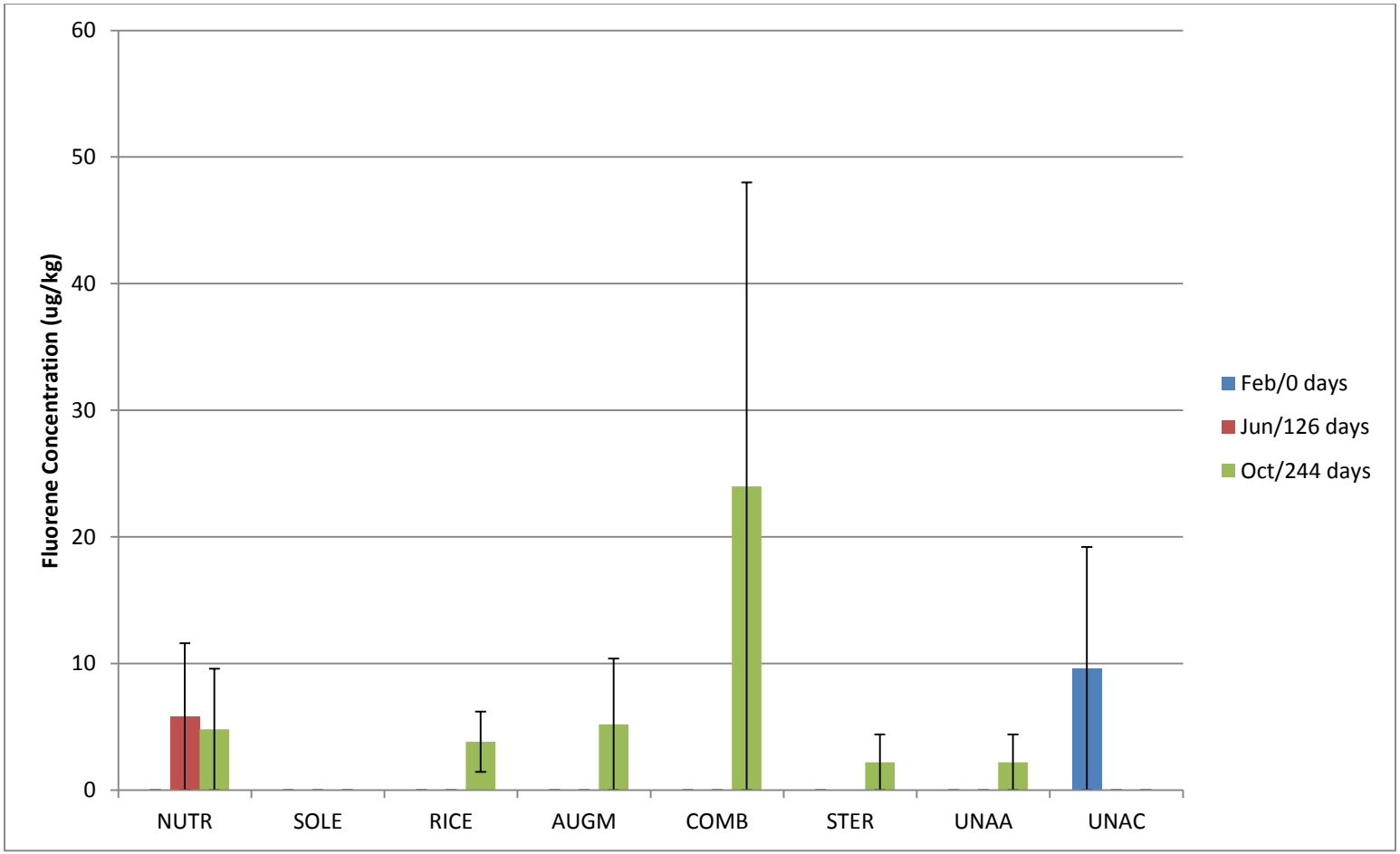

Figure F-21: Fluorene concentrations during microcosm incubation (Soils A and C)

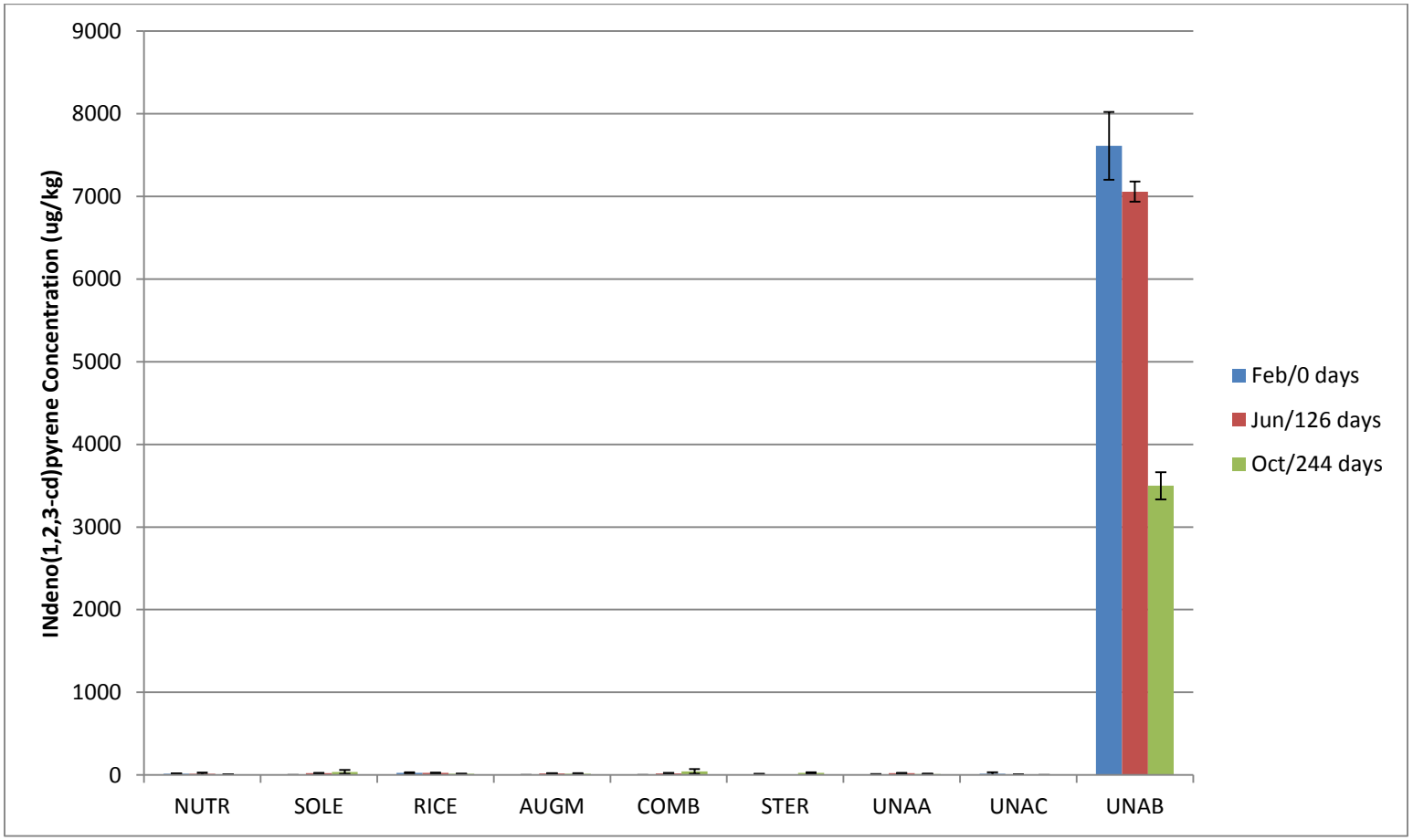

Figure F-22: Indeno(1,2,3-cd)pyrene concentrations during microcosm incubation (all soils)

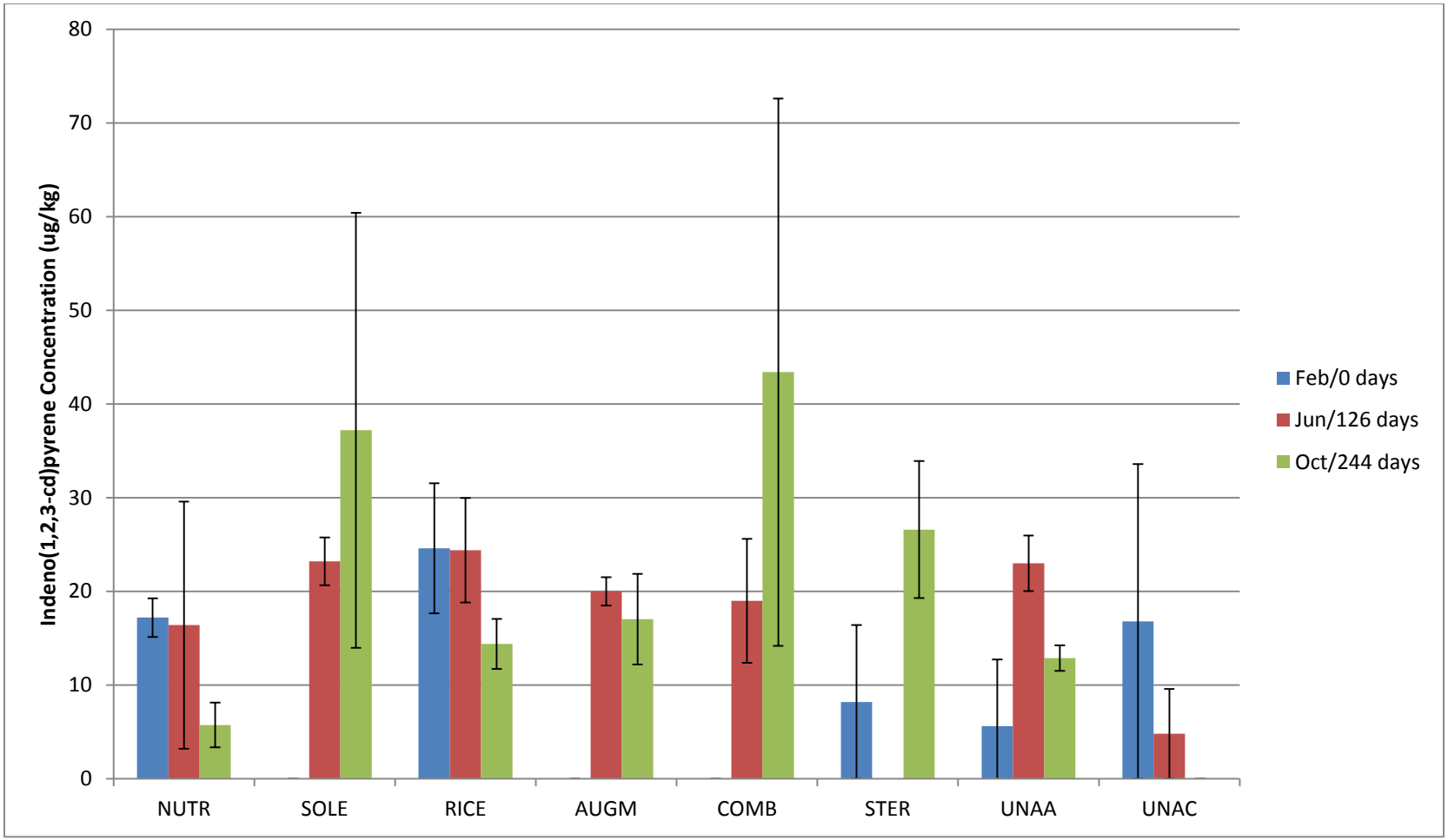

Figure F-23: Indeno(1,2,3-cd)pyrene concentrations during microcosm incubation (Soils A and C)

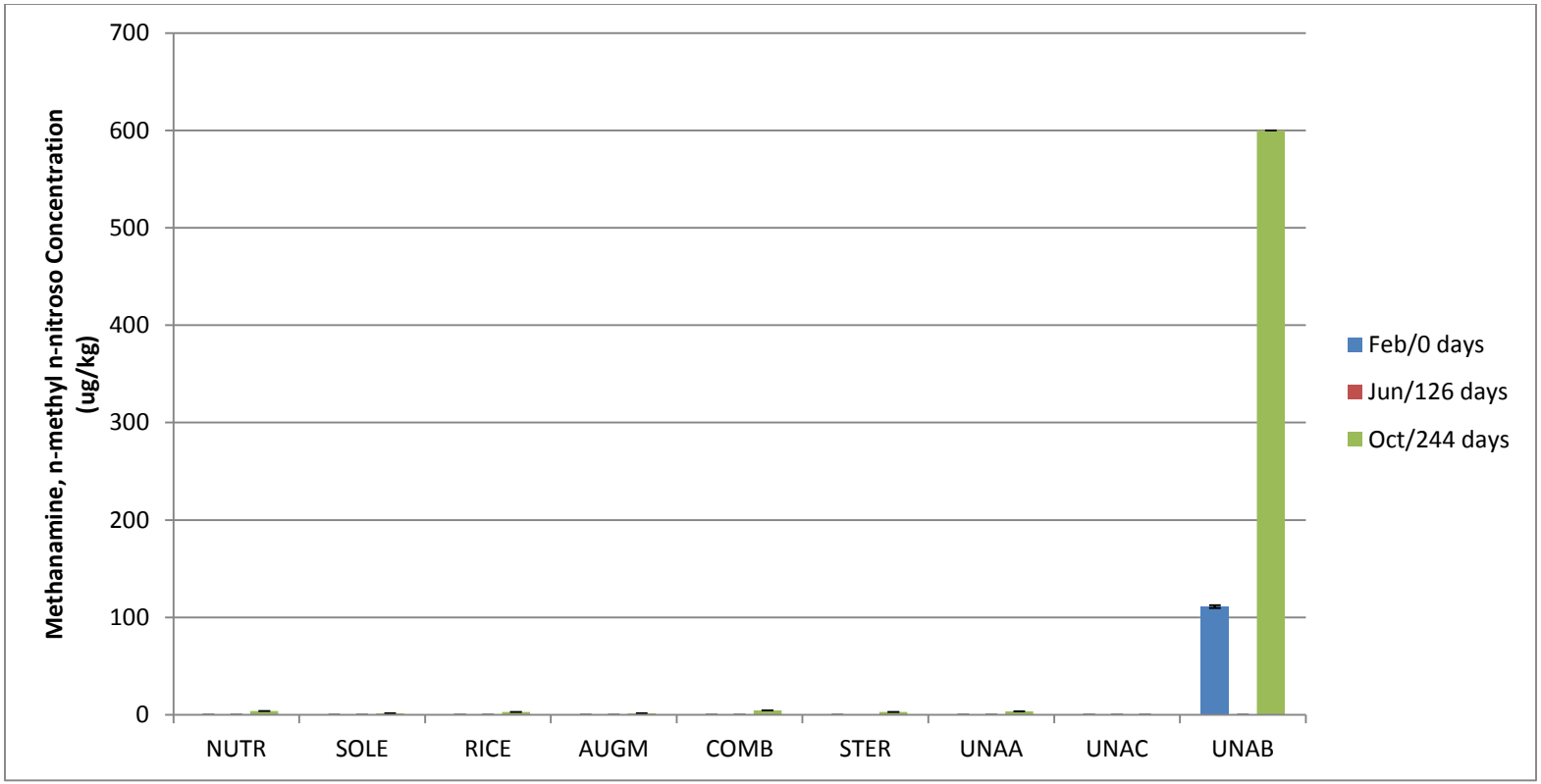

Figure F-24: Methanamine, n-methyl n-nitroso concentrations during microcosm incubation (all soils)

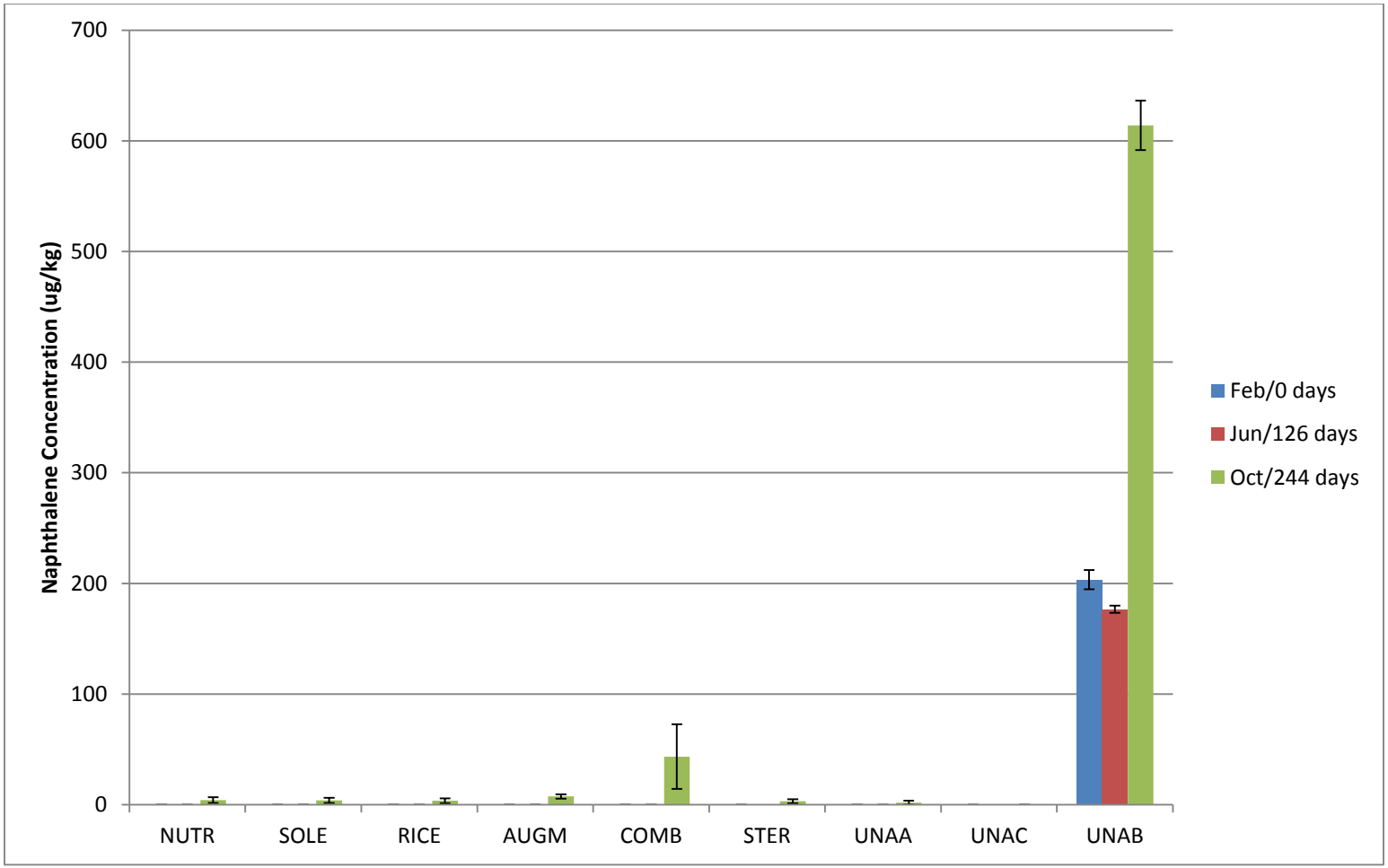

Figure F-25: Naphthalene concentrations during microcosm incubation (all soils)

Appendix G: Microcosm Results for PCB Aroclor Concentrations

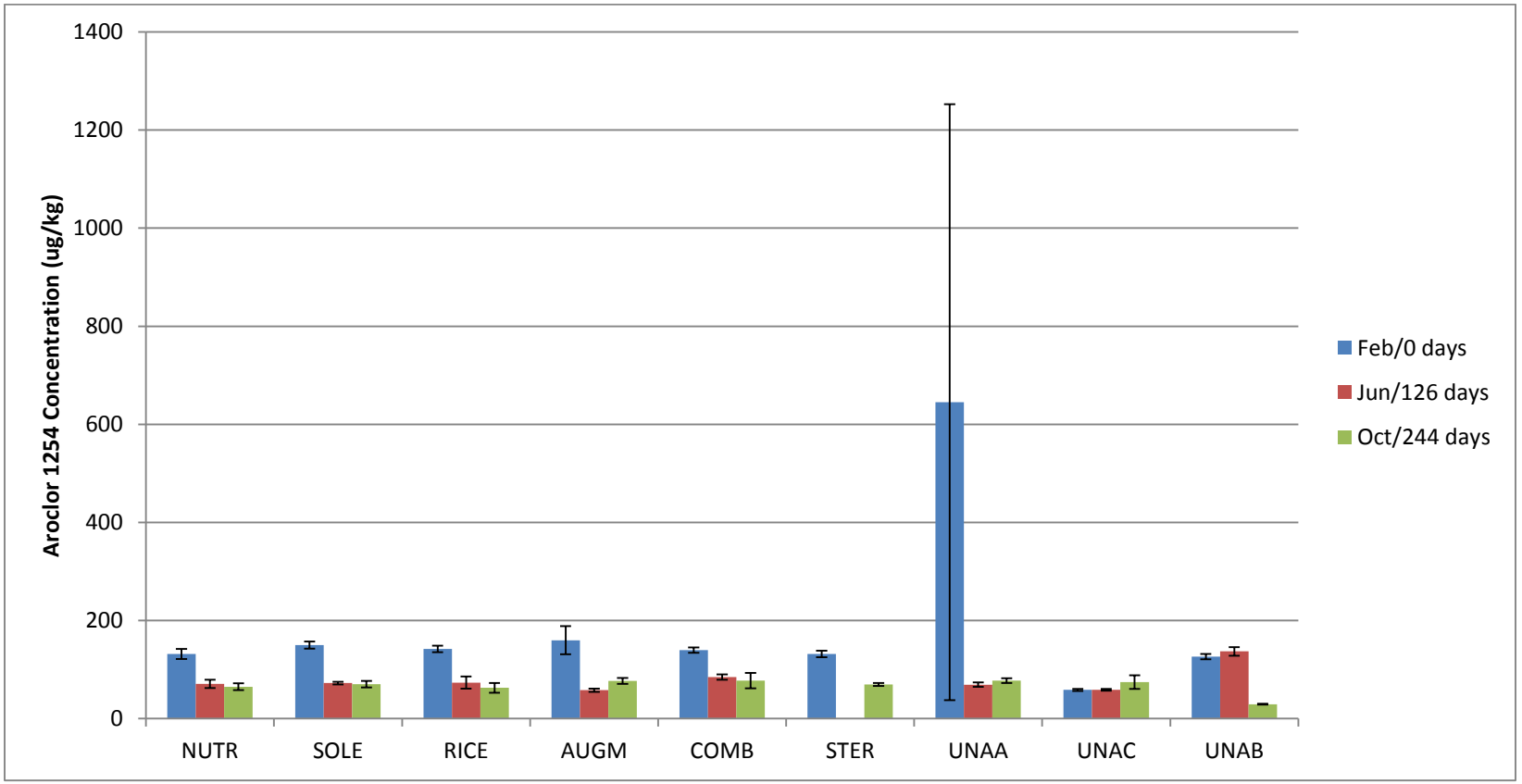

Figure G-1: Aroclor 1254 concentration during microcosm incubation

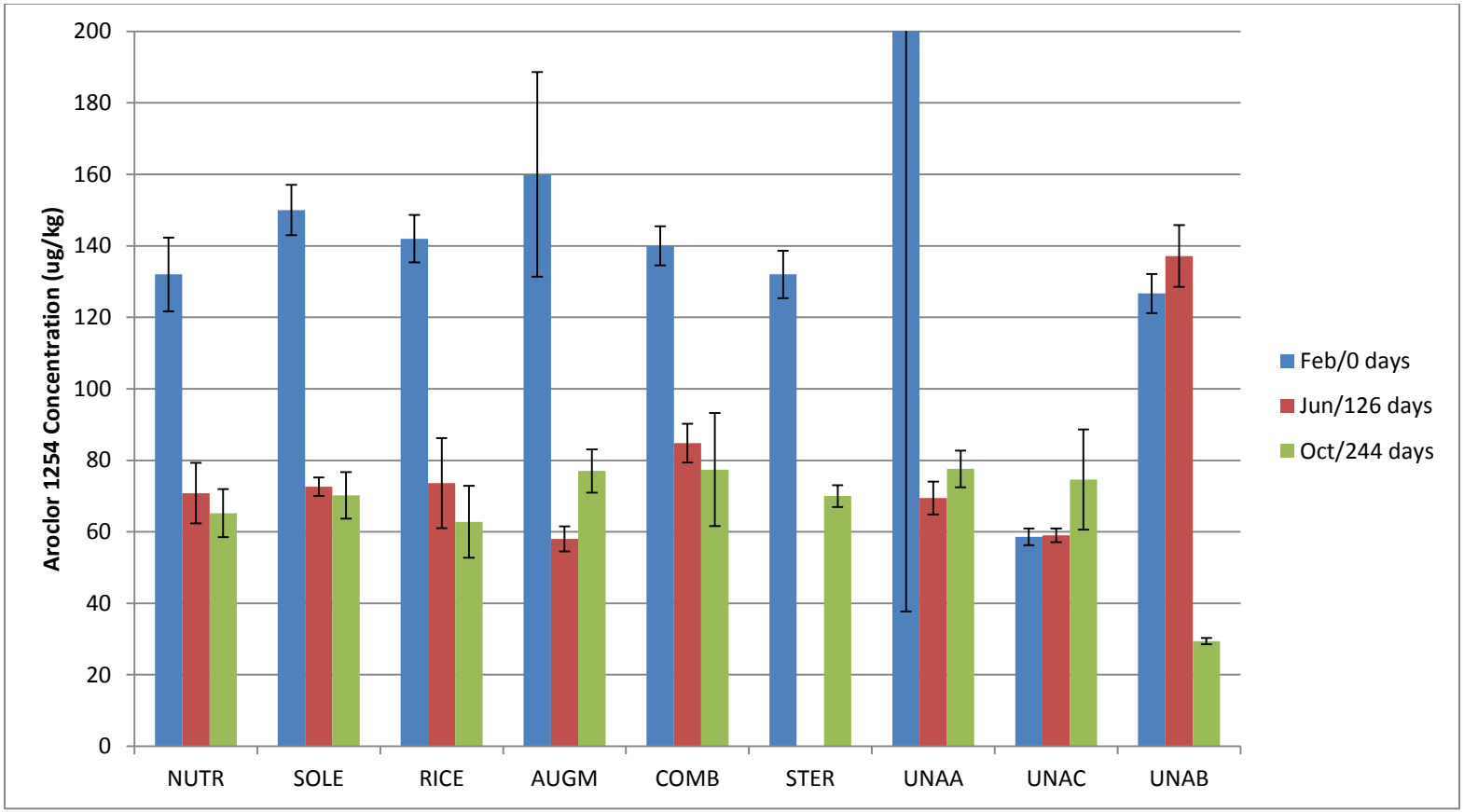

Figure G-2: Truncated Aroclor 1254 concentrations during microcosm incubation

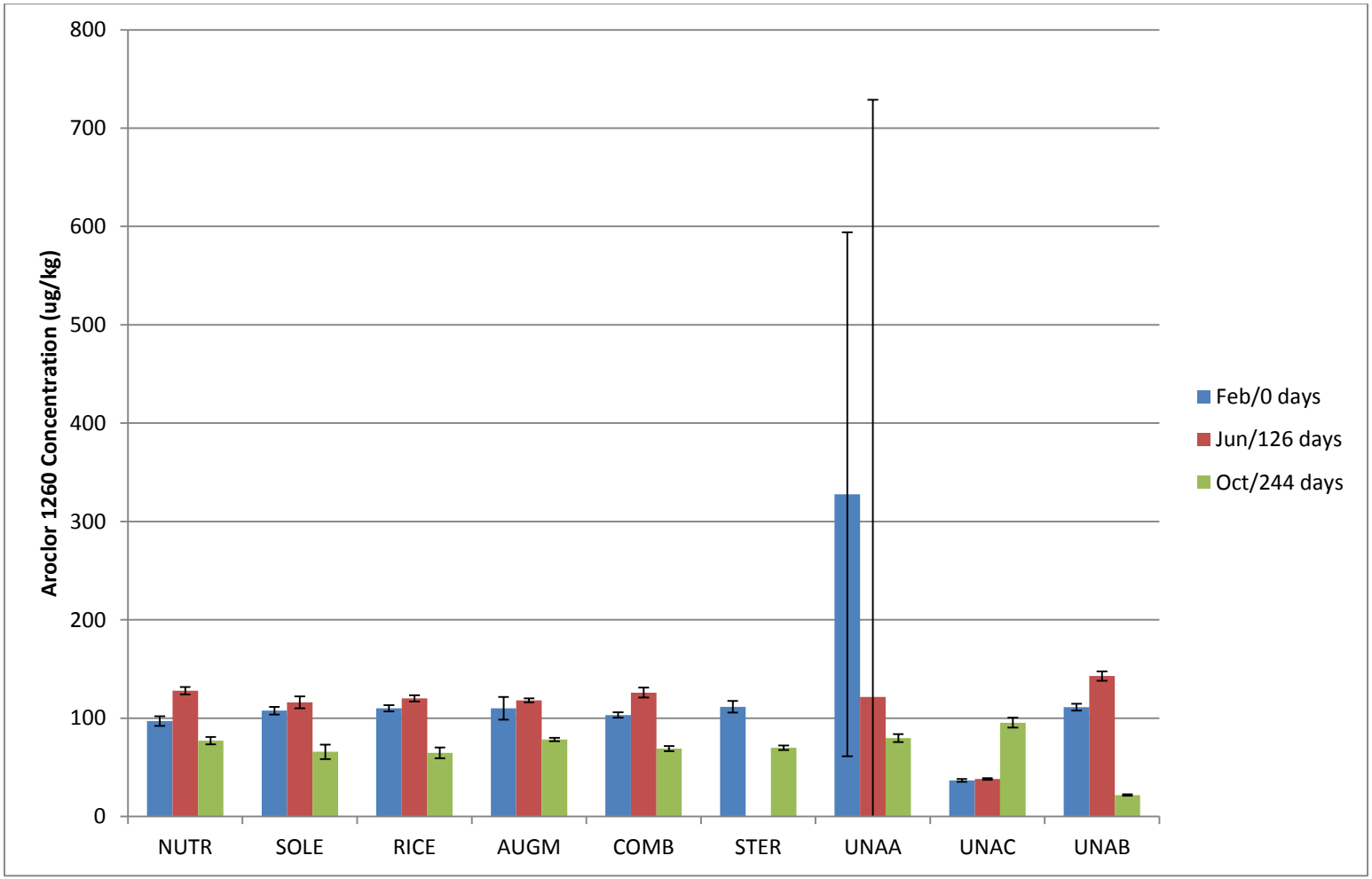

Figure G-3: Aroclor 1260 concentrations during microcosm incubation

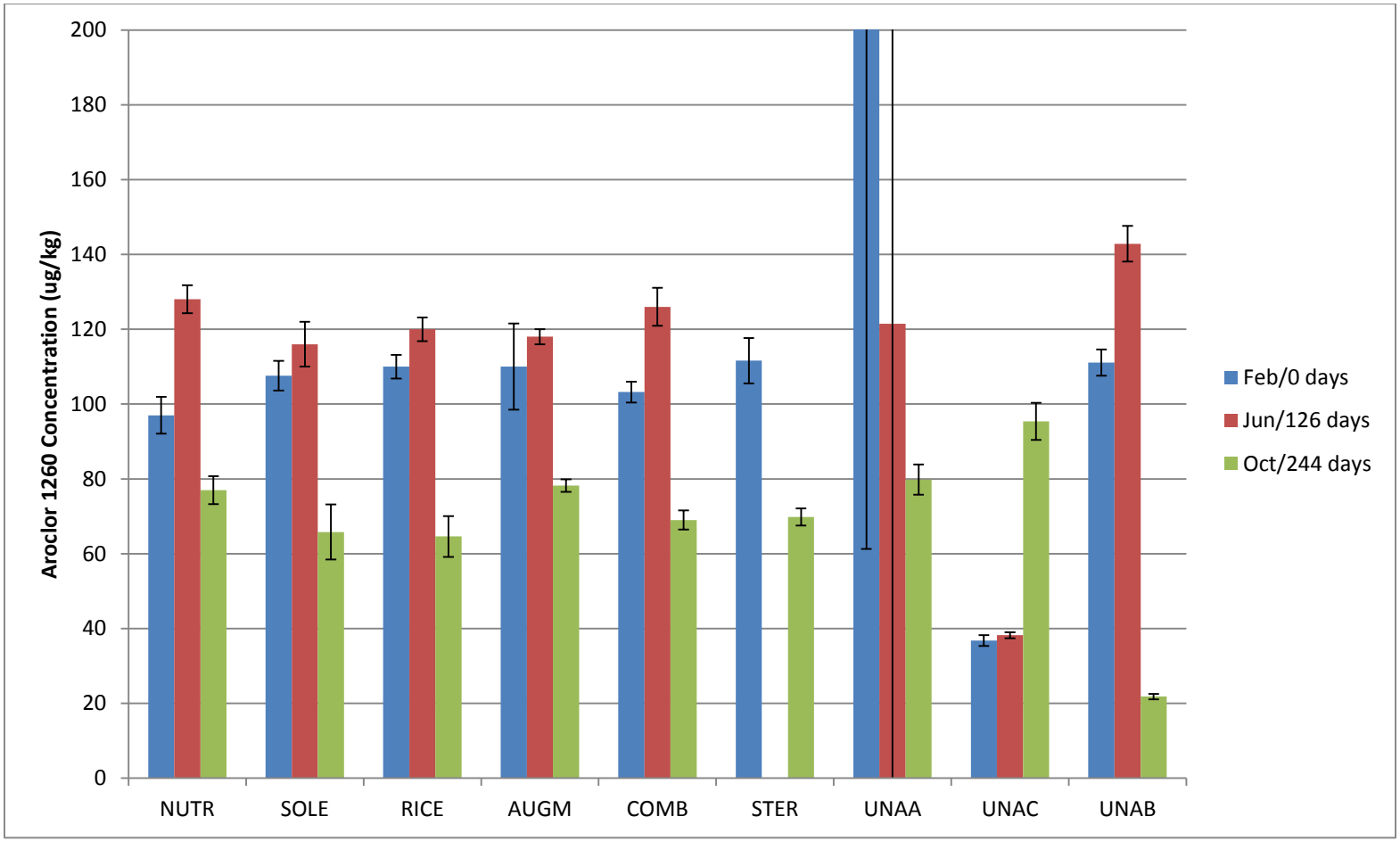

Figure G-4: Truncated Aroclor 1260 concentrations during microcosm incubation

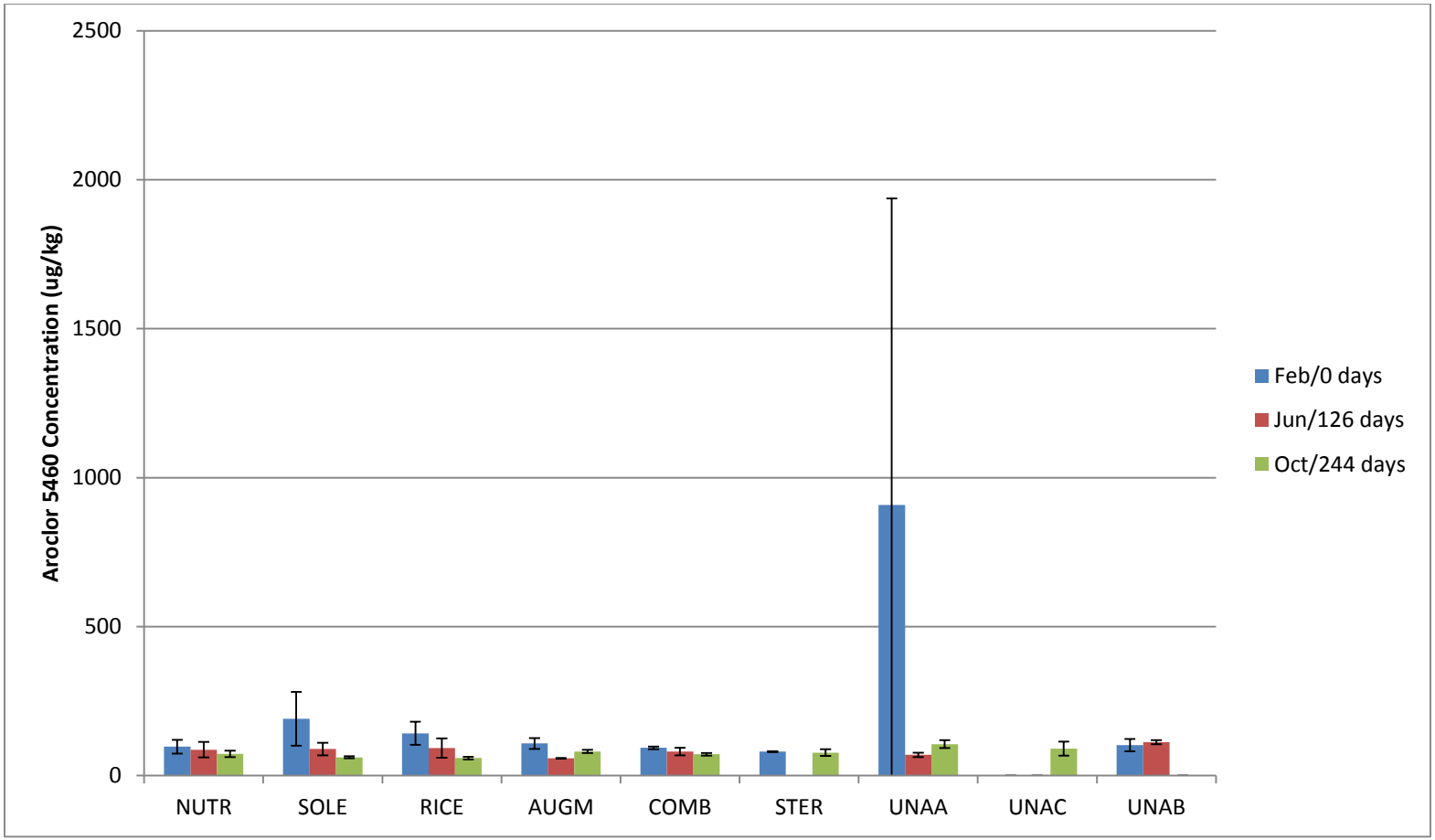

Figure G-5: Aroclor 5460 concentrations during microcosm incubation

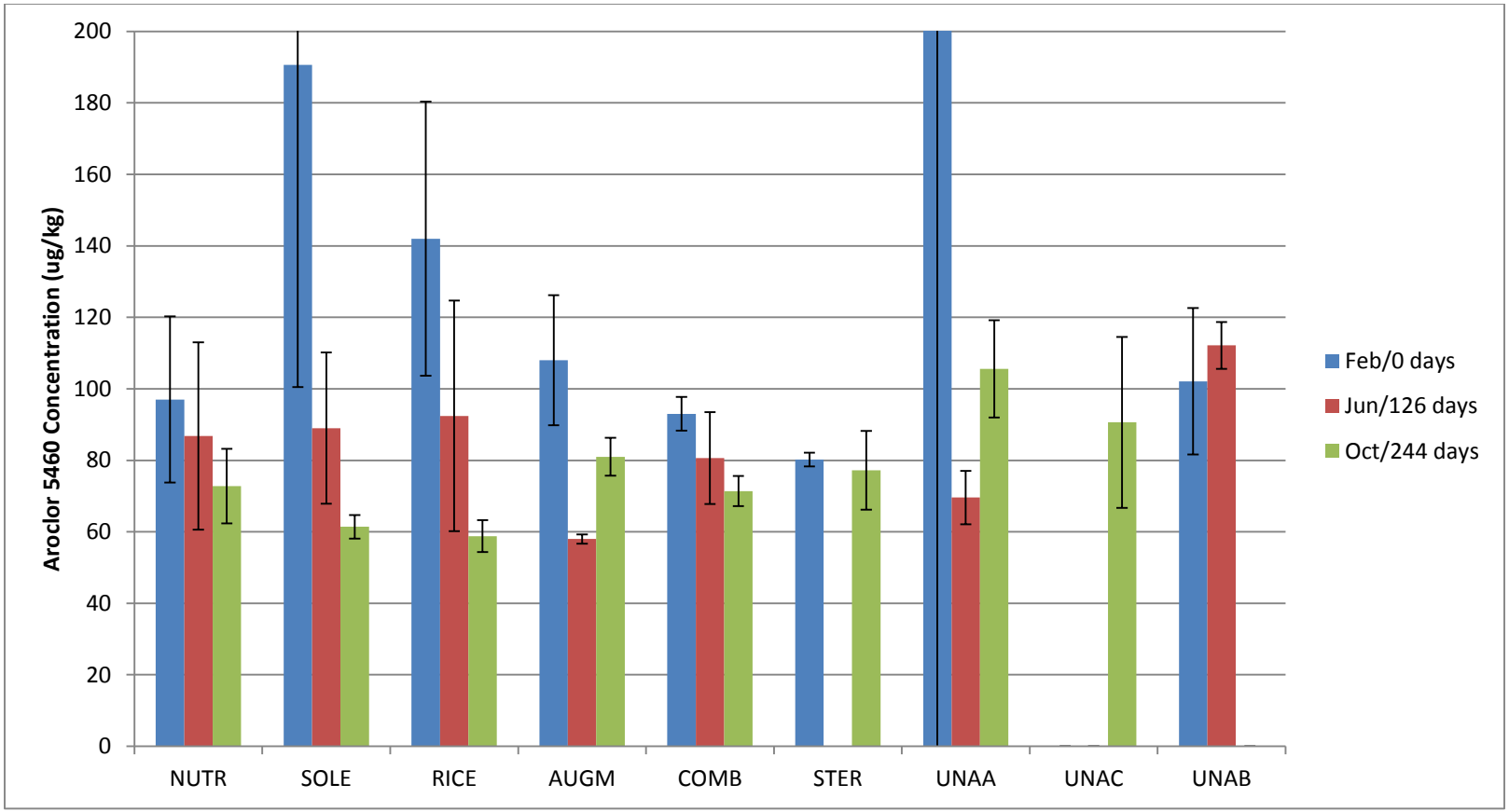

Figure G-6: Truncated Aroclor 5460 concentrations during microcosm incubation

Appendix H: Microcosm Data and Graphs

Table H-1: Microcosm soil pH values and statistics

Microcosm ID/Type	***Average Value***		***Standard Deviation***		***Standard Error***	
Sampling time (month/days)	Feb/0 days	Jun/126 days	Feb/0 days	Jun/126 days	Feb/0 days	Jun/126 days
A1 Nutrients	6.53	6.28	0.04	0.06	0.02	0.03
A2 Soya lecithin	5.87	6.31	0.03	0.19	0.01	0.08
A3 Rice hulls	6.60	6.24	0.07	0.17	0.03	0.08
A4 Nutrients+rice hulls+*P. chrysosporium*	6.44	6.35	0.06	0.18	0.03	0.08
A5 Nutrients+soya lecithin+rice hulls+*P. chrysosporium*	6.03	6.18	0.03	0.09	0.01	0.04
A6 Unamended Soil A	6.64	6.30	0.03	0.02	0.01	0.01
B6 Unamended Soil B	6.84	6.68	0.03	0.05	0.01	0.02
C6 Unamended Soil C	7.35	7.33	0.05	0.05	0.02	0.02
A7 Unamended, gamma-irradiated Soil A	6.676	N/A	0.038471	N/A	0.017205	N/A

Table H-2: Microcosm soil TOC concentrations and statistics

Microcosm ID/Type	***Average Concentration (mg/kg)***		***Standard Deviation***		***Standard Error***	
Sampling time (month/days)	Feb/0 days	Jun/126 days	Feb/0 days	Jun/126 days	Feb/0 days	Jun/126 days
A1 nutrient	11976	7986	7266	2594	3250	1160
A2 soya lecithin	24960	12524	9630	2892	4307	1294
A3 rice hulls	36420	23820	17833	6181	7975	2764
A4 nutrients+rice hulls+P. chrysosporium	33464	19840	22393	3331	10015	1490
A5 nutrients+soya lecithin+rice hulls+P. chrysosporium	37640	23740	11336	4426	5070	1979
A6 unamended Soil A	11298	9268	5606	3056	2507	1367
B6 unamended Soil B	61060	37530	19453	28015	8700	12529
C6 unamended Soil C	3236	2770	1023	1231	458	551
A7 gamma-irradiated unamended Soil A	10830	N/A	3732	N/A	1669	N/A

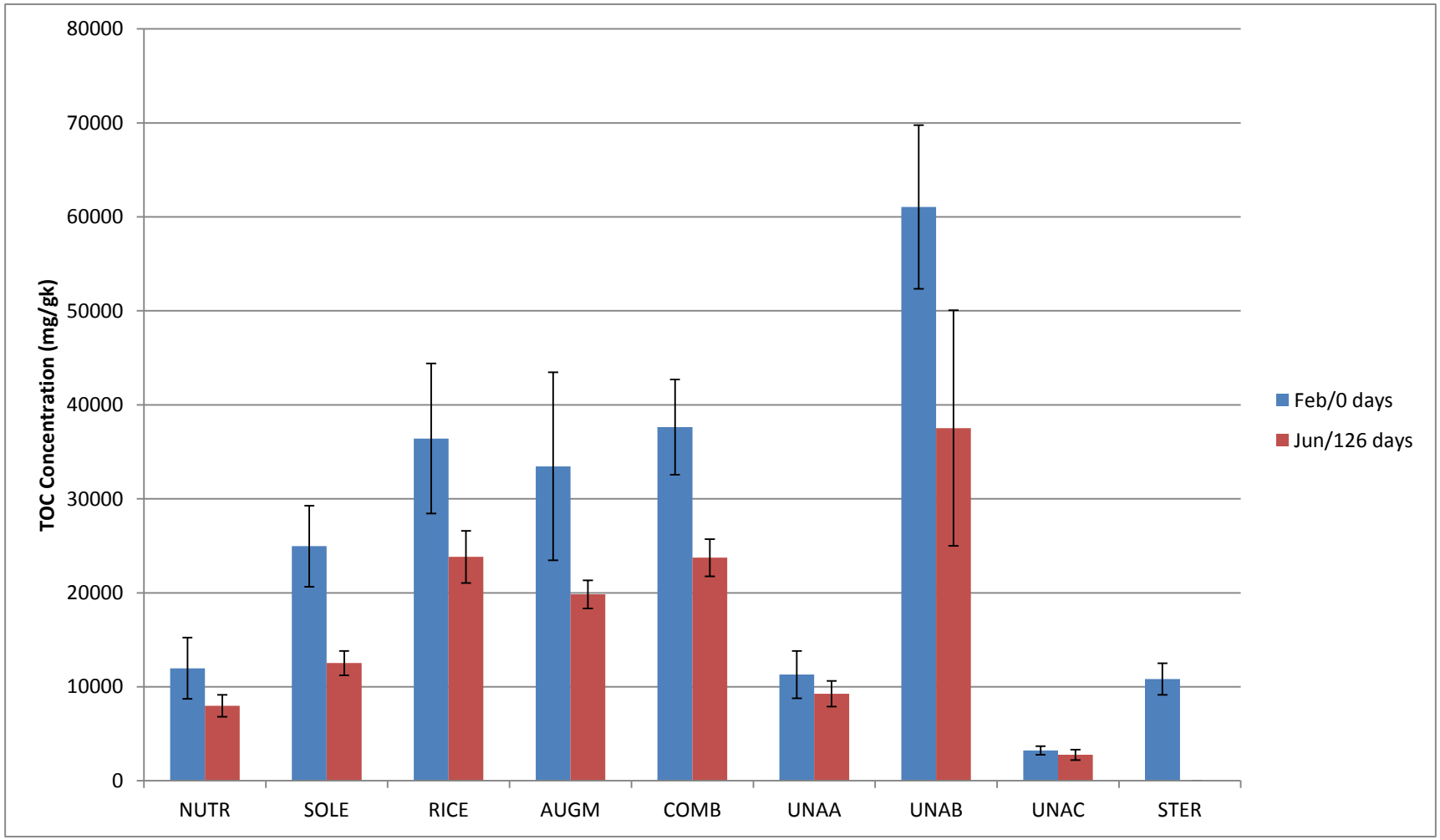

Figure H-1: TOC in microcosms during incubation

Table H-3: Microcosm soil Nitrate/Nitrate concentrations and statistics

Microcosm ID/Type	*Average Concentration (mg/kg)*		*Standard Deviation*		*Standard Error*	
Sampling time (month/days)	**Feb/0 days**	**Jun/126 days**	**Feb/0 days**	**Jun/126 days**	**Feb/0 days**	**Feb/0 days**
A1 nutrient	701	991	146	126	65	57
A2 soya lecithin	772	1148	104	138	47	62
A3 rice hulls	856	1066	181	657	81	294
A4 nutrients+rice hulls+P. chrysosporium	884	1103	464	149	207	66
A5 nutrients+soya lecithin+rice hulls+P. chrysosporium	996	1584	117	454	52	203
A6 unamended Soil A	834	1168	198	318	88	142
B6 unamended Soil B	1814	2178	437	229	195	103
C6 unamended Soil C	186	143	41	82	18	37
A7 gamma-irradiated unamended Soil A	679	N/A	77	N/A	34	N/A

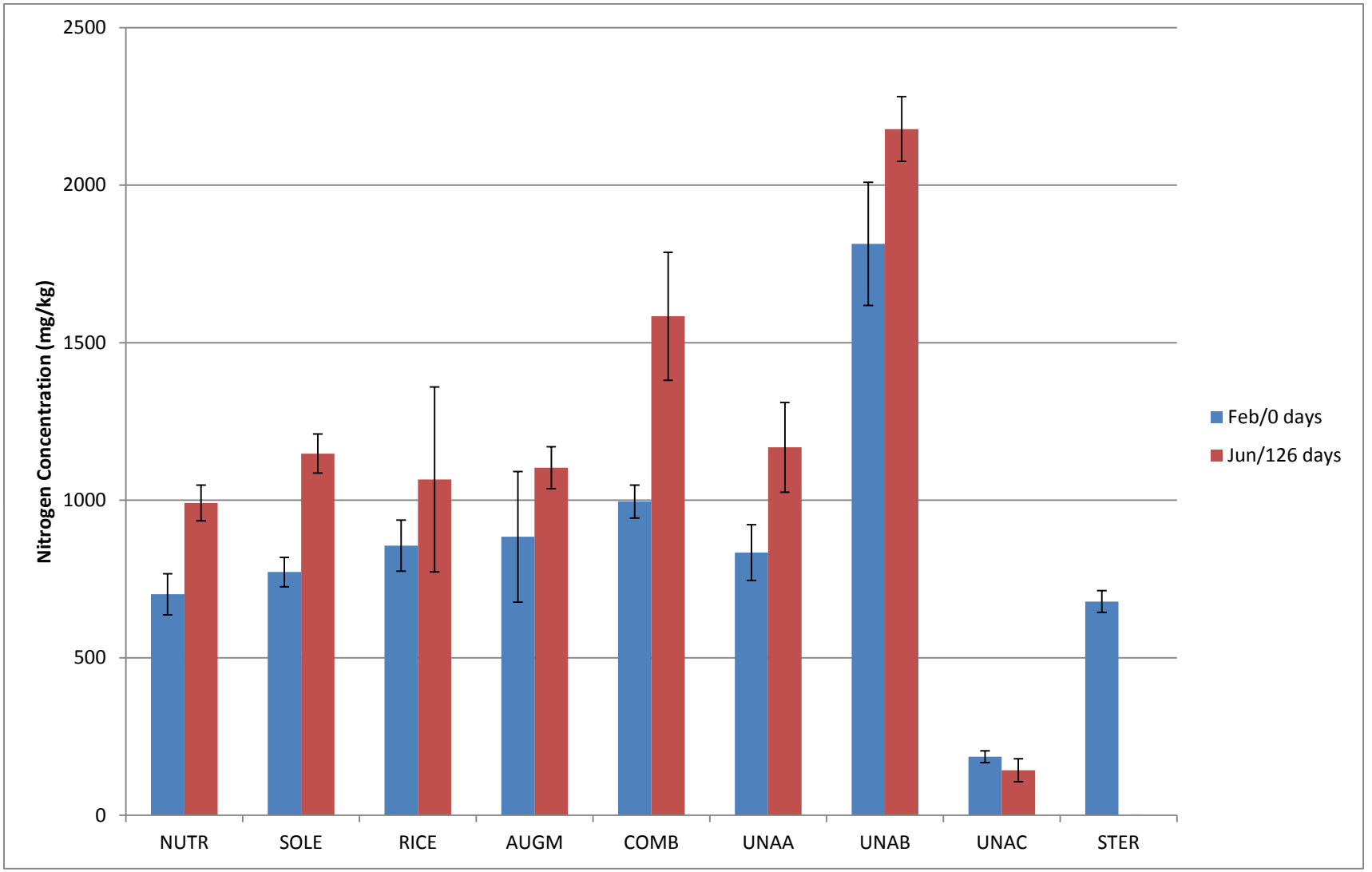

Figure H-2: Nitrogen in microcosms during incubation

Table H-4: Microcosm soil moisture content and statistics

Microcosm ID/Type	***Average moisture content (%)***			***Standard Deviation***			***Standard Error***		
Sampling time (month/day)	Feb/0	Jun/126	Oct/244	Feb/0	Jun/126	Oct/244	Feb/0	Jun/126	Oct/244
A1 nutrient	11.7	11.6	15.6	0.3	1.5	1.6	0.2	0.7	0.7
A2 soya lecithin	11.3	13.2	15.5	2.6	0.9	0.8	1.1	0.4	0.4
A3 rice hulls	8.9	12.4	15.4	1.0	1.2	0.5	0.4	0.5	0.2
A4 nutrients+rice hulls+P. chrysosporium	12.2	13.0	15.3	1.3	1.5	1.6	0.6	0.7	0.7
A5 nutrients+soya lecithin+rice hulls+P. chrysosporium	12.8	12.2	16.8	1.0	0.7	1.7	0.4	0.3	0.7
A6 unamended Soil A	11.9	11.5	14.4	0.6	0.6	0.3	0.3	0.3	0.1
B6 unamended Soil B	12.2	11.1	14.9	1.3	0.9	0.9	0.6	0.4	0.4
C6 unamended Soil C	11.6	12.2	15.5	0.4	0.9	1.8	0.2	0.4	0.8
A7 gamma-irradiated unamended Soil A	11.4	N/A	11.5	0.8	N/A	0.4	0.3	N/A	0.2

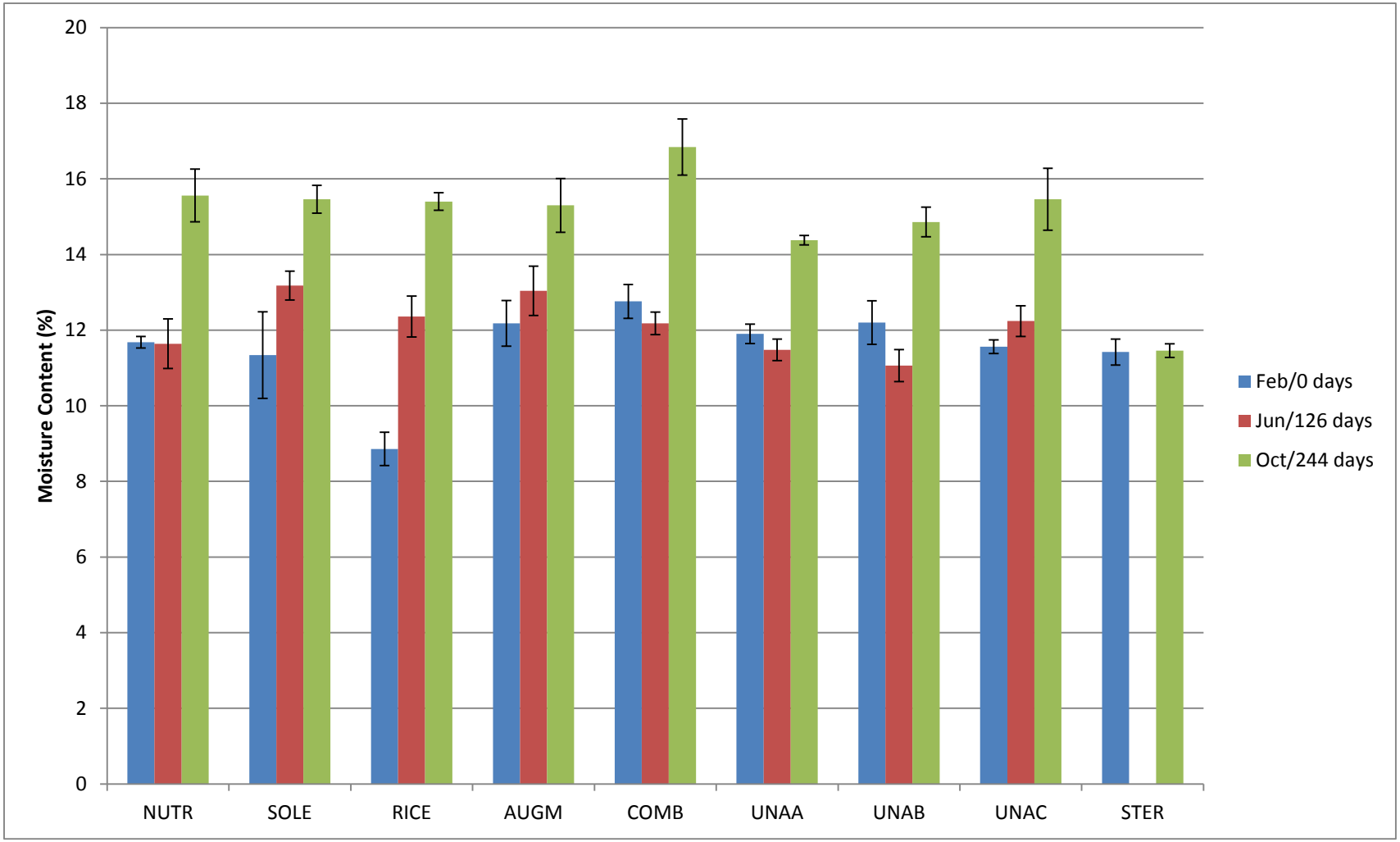

Figure H-3: Microcosm moisture content during incubation

Table H-5: Microcosm soil total EFH concentrations and statistics

Microcosm ID/type	*Average Concentration (mg/kg)*			*Standard Deviation*			*Standard Error*		
Sampling time (month/day)	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**
A1 nutrient	182	152	152	51	54	119	23	24	53
A2 soya lecithin	1640	172	N/A	219	54	N/A	98	24	N/A
A3 rice hulls	154	137	502	22	46	502	9	20	225
A4 nutrients+rice hulls+P. chrysosporium	146	113	556	23	27	556	10	12	249
A5 nutrients+soya lecithin+rice hulls+P. chrysosporium	1980	156	N/A	327	32	N/A	146	14	N/A
A6 unamended Soil A	152	89	502	55	12	79	25	5	35
B6 unamended Soil B	230	226	1589	12	30	188	5	14	84
C6 unamended Soil C	100	105	558	29	26	54	13	12	24
A7 gamma-irradiated unamended Soil A	101	N/A	628	9	N/A	42	4	N/A	19

Table H-6: Microcosm total PAH concentrations and statistics

Microcosm ID/type	*Average Concentration (ug/kg)*			*Standard Deviation*			*Standard Error*		
Sampling time (month/day)	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**
A1 nutrient	626	727	673	228	881	714	102	394	319
A2 soya lecithin	350	538	1390	191	195	1812	85	87	810
A3 rice hulls	714	489	759	414	282	372	185	126	166
A4 nutrients+rice hulls+P. chrysosporium	214	382	710	39	113	479	15	50	214
A5 nutrients+soya lecithin+rice hulls+P. chrysosporium	87	485	1672	56	282	2014	25	126	901
A6 unamended Soil A	467	429	684	297	158	224	133	71	100
B6 unamended Soil B	45139	39238	40585	3441	1746	5198	1539	781	2325
C6 unamended Soil C	626	153	50	1361	211	50	609	94	23
A7 gamma-irradiated unamended Soil A	523	N/A	943	364	N/A	289	163	N/A	129

Table H-7: Microcosm soil total PCB concentrations and statistics

Microcosm ID/type	*Average Concentration (µg/kg)*			*Standard Deviation*			*Standard Error*		
Sampling time (month/day)	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**
A1 nutrient	326.4	285.6	215	81.36523	54.6562	37.22902	36.38764	24.44299	16.64932
A2 soya lecithin	448.2	277.6	197.4	214.9353	46.59184	34.07785	96.12201	20.83651	15.24008
A3 rice hulls	394	286	186.2	98.66357	104.8308	43.47643	44.12369	46.88177	19.44325
A4 nutrients+rice hulls+P. chrysosporium	378	234	240.2	101.4569	10.41633	19.17551	45.3729	4.658326	8.575547
A5 nutrients+soya lecithin+rice hulls+P. chrysosporium	336.2	291.4	217.8	19.46022	43.51781	44.81852	8.702873	19.46176	20.04345
A6 unamended Soil A	2811.8	251.4	263	5362.195	17.7426	49.86482	2398.047	7.934734	22.30022
B6 unamended Soil B	329.2	414	260.6	37.66563	33.61547	30.66431	16.84458	15.0333	13.7135
C6 unamended Soil C	95.4	97.2	51.2	8.414274	5.674504	3.563706	3.762978	2.537716	1.593738
A7 gamma-irradiated unamended Soil A	323.8		217	27.98571		30.8788	12.51559		13.80942

Table H-8: Microcosm total dioxin concentration and statistics

Microcosm ID/type	*Average Concentration (ng/kg)*			*Standard Deviation*			*Standard Error*		
Sampling time (month/day)	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**
A1 nutrient	98898	79230	126710	11141	3779	57719	4982	1690	25813
A2 soya lecithin	99547	84227	88048	6749	14579	4316	3018	6520	1930
A3 rice hulls	89064	85548	93723	6327	19916	11257	2830	8907	5034
A4 nutrients+rice hulls+P. chrysosporium	116316	90113	85415	51418	20613	85415	22995	9219	38199
A5 nutrients+soya lecithin+rice hulls+P. chrysosporium	100358	88368	97854	13966	12666	17633	6246	5665	7886
A6 unamended Soil A	99432	81967	96257	9032	2047	19335	4039	915	8647
B6 unamended Soil B	26581	26041	30452	1536	2396	2397	687	1072	1072
C6 unamended Soil C	54509	54526	55342	7608	6219	12275	3403	2781	5490
A7 gamma-irradiated unamended Soil A	91803		99035	18189		7052	8135		3154

Table H-9: Microcosm soil TCDD TEQ concentrations and statistics

Microcosm ID/type	*Average Concentration (ng/kg)*			*Standard Deviation*			*Standard Error*		
Sampling time (month/day)	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**	**Feb/0 days**	**Jun/126 days**	**Oct/244 days**
A1 nutrient	297	247	247	28	20	137	13	9	61
A2 soya lecithin	303	264	264	27	27	10	12	12	4
A3 rice hulls	267	250	276	18	15	28	8	7	12
A4 nutrients+rice hulls+P. chrysosporium	332	266	282	137	34	11	61	15	5
A5 nutrients+soya lecithin+rice hulls+P. chrysosporium	286	262	314	33	19	41	15	8	18
A6 unamended Soil A	288.4	263.8	308.8	12.66096	4.32435	29.72709	5.662155	1.933908	13.29436
B6 unamended Soil B	57.22	53.92	66.98	2.277499	2.20159	4.350517	1.018528	0.984581	1.94561
C6 unamended Soil C	55.14	56.24	62.38	6.518666	4.646827	10.97802	2.915236	2.078124	4.909521
A7 gamma-irradiated unamended Soil A	266		313.8	17.50714		8.074652	7.829432		3.611094

Appendix I: Individual Compound Concentration (Mean) and Total EFH, PAH, PCB, and Dioxin Concentrations during Microcosm Experiment (Including Standard Deviation and Standard Error of the Mean in lower parts of the table)

Table I-1: Individual compound concentrations during microcosm experiment including standard deviation and error

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
Feb/0 days										
Chemical	Units	Mean Concentration								
1,2,3,4,6,7,8-HEPTACHLORODIBENZO-P-DIOXIN	ng/kg	10510	11740	9756	11382	10826	9078	9953	2798	2083
1,2,3,4,6,7,8-HPCDF	ng/kg	983	861	818	1249	979	815	870	176	255
1,2,3,4,7,8,9-HPCDF	ng/kg	84	76	72	88	68	69	75	10	19
1,2,3,4,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	83	83	74	92	79	75	81	5	16
1,2,3,4,7,8-HXCDF	ng/kg	25	22	22	31	24	22	24	3	10
1,2,3,6,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	478	484	429	523	441	430	444	61	80
1,2,3,6,7,8-HXCDF	ng/kg	31	25	25	55	44	24	26	3	9
1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	189	190	169	205	173	169	178	14	34
1,2,3,7,8,9-HXCDF	ng/kg	7	6	6	8	2	4	3	0	3
1,2,3,7,8-PENTACHLORODIBENZOFURAN	ng/kg	8	8	8	9	7	7	8	1	3
1,2,3,7,8-PENTACHLORODIBENZO-P-DIOXIN	ng/kg	56	53	50	62	50	53	55	2	8

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
2,3,4,6,7,8-HXCDF	ng/kg	49	44	42	54	41	42	45	3	13
2,3,4,7,8-PECDF	ng/kg	12	10	10	13	10	10	11	1	7
2,3,7,8-TCDD	ng/kg	9	9	8	10	8	9	9	0	1
2,3,7,8-TETRACHLORODIBENZOFURAN	ng/kg	3	2	2	3	2	2	2	1	4
OCDD	ng/kg	84340	84080	75840	100420	85980	79280	84738	50860	22600
OCDF	ng/kg	2034	1854	1734	2112	1626	1714	1794	572	835
TCDD TEQ	ng/kg	297	303	267	332	286	266	288	55	57
1,1'-Biphenyl	µg/kg	0	0	0	0	0	0	0	0	62
1-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0	0	0	0	0
2-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0	0	0	0	138
ACENAPHTHENE	µg/kg	0	0	0	0	0	0	0	16	0
ACENAPHTHYLENE	µg/kg	0	0	8	0	0	0	0	0	151
ANTHRACENE	µg/kg	7	0	9	0	0	0	5	22	639
AZOBENZENE	µg/kg	0	0	0	0	0	0	0	0	0
BENZO(A)ANTHRACENE	µg/kg	39	21	20	0	0	19	27	38	1322
BENZO(A)PYRENE	µg/kg	38	24	48	12	0	38	37	44	4500
BENZO(B)FLUORANTHENE	µg/kg	66	55	69	35	0	67	69	54	6278
Benzo(e)pyrene	µg/kg	49	40	61	26	12	47	53	42	4356
BENZO(G,H,I)PERYLENE	µg/kg	27	18	58	34	0	38	35	20	7922
BENZO(K)FLUORANTHENE	µg/kg	16	0	7	0	0	6	0	18	1157
bis(2-Ethylhexyl)phthalate	µg/kg	0	0	0	0	0	0	0	0	0
Butylbenzylphthalate	µg/kg	0	0	0	0	0	0	0	0	0
Chrysene	µg/kg	68	40	57	0	0	43	53	50	2256
Di-n-butylphthalate	µg/kg	0	0	0	0	0	0	0	0	0
DIBENZO(A,H)ANTHRACENE	µg/kg	0	0	0	0	0	0	0	6	1211
Di-n-octylphthalate	µg/kg	0	0	0	0	0	0	0	0	0
FLUORANTHENE	ug/kg	129	86	144	49	62	102	110	96	2622
FLUORENE	µg/kg	0	0	0	0	0	0	0	10	84

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
INDENO(1,2,3-CD)PYRENE	ug/kg	17	0	25	0	0	8	6	17	7611
METHANAMINE, N-METHYL-N-NITROSO	µg/kg	0	0	0	0	0	0	0	0	111
NAPHTHALENE	µg/kg	0	0	0	0	0	0	0	0	203
PHENANTHRENE	µg/kg	55	47	92	16	0	61	38	100	2222
PYRENE	µg/kg	114	20	117	42	24	93	94	94	1678
Aroclor 1016	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1221	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1232	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1242	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1248	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1254	µg/kg	132	150	142	160	140	132	645	59	127
Aroclor 1260	µg/kg	97	108	110	110	103	112	328	37	111
Aroclor 1262	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1268	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5432	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5442	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5460	µg/kg	97	191	142	108	93	80	908	0	102
EFH (C12-C14)	mg/kg	0	21	0	0	1	0	1	0	1
EFH (C15-C20)	mg/kg	0	376	4	5	404	0	2	0	17
EFH (C21-C30)	mg/kg	94	1046	79	84	1414	55	79	49	133
EFH (C30-C40)	mg/kg	87	166	73	57	130	45	52	50	86
EFH (C8-C11)	mg/kg	0	14	0	0	1	0	1	0	1
Jun/126 days										
Chemical	Units	Mean Concentration								
1,2,3,4,6,7,8-HEPTACHLORODIBENZO-P-DIOXIN	ng/kg	9292	9774	9186	10120	9850	N/A	9571	2956	2171
1,2,3,4,6,7,8-HPCDF	ng/kg	781	790	789	821	850	N/A	828	167	252

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
1,2,3,4,7,8,9-HPCDF	ng/kg	70	70	68	71	72	N/A	72	12	19
1,2,3,4,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	72	73	69	73	70	N/A	74	5	16
1,2,3,4,7,8-HXCDF	ng/kg	21	21	21	22	23	N/A	22	2	10
1,2,3,6,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	411	427	402	406	427	N/A	432	60	82
1,2,3,6,7,8-HXCDF	ng/kg	23	23	23	23	25	N/A	25	2	8
1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	169	172	158	172	167	N/A	176	13	35
1,2,3,7,8,9-HXCDF	ng/kg	5	7	3	4	4	N/A	2	0	0
1,2,3,7,8-PENTACHLORODIBENZOFURAN	ng/kg	7	7	7	7	7	N/A	7	0	3
1,2,3,7,8-PENTACHLORODIBENZO-P-DIOXIN	ng/kg	49	50	46	48	48	N/A	52	2	8
2,3,4,6,7,8-HXCDF	ng/kg	42	41	40	42	42	N/A	42	4	13
2,3,4,7,8-PECDF	ng/kg	8	10	9	9	9	N/A	9	1	7
2,3,7,8-TCDD	ng/kg	9	9	8	8	9	N/A	8	0	1
2,3,7,8-TETRACHLORODIBENZOFURAN	ng/kg	2	2	2	2	2	N/A	2	1	4
OCDD	ng/kg	66580	71080	76000	76500	74900	N/A	68871	50720	23129
OCDF	ng/kg	1688	1672	13584	1786	1864	N/A	1804	580	831
TCDD TEQ	ng/kg	247	264	534	266	262	N/A	264	56	56
1,1'-Biphenyl	µg/kg	0	0	0	0	0	N/A	0	0	48
1-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0	N/A	0	0	67
2-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0	N/A	0	0	103
ACENAPHTHENE	µg/kg	9	0	0	0	0	N/A	0	0	12
ACENAPHTHYLENE	µg/kg	0	3	0	0	0	N/A	0	0	129
ANTHRACENE	µg/kg	16	6	3	7	0	N/A	0	0	566
AZOBENZENE	µg/kg	0	0	0	0	0	N/A	0		0
BENZO(A)ANTHRACENE	µg/kg	46	27	24	8	30	N/A	23	16	1171
BENZO(A)PYRENE	µg/kg	49	29	36	26	36	N/A	33	6	3757

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
BENZO(B)FLUORANTHENE	µg/kg	75	59	58	45	60	N/A	58	17	5043
Benzo(e)pyrene	µg/kg	49	34	37	35	44	N/A	40	33	3514
BENZO(G,H,I)PERYLENE	µg/kg	36	29	36	33	28	N/A	35	15	7057
BENZO(K)FLUORANTHENE	µg/kg	13	13	11	5	15	N/A	10	3	1060
bis(2-Ethylhexyl)phthalate	µg/kg	0	0	0	0	0	N/A	0	0	0
Butylbenzylphthalate	µg/kg	0	0	0	0	0	N/A	0	0	0
Chrysene	µg/kg	68	60	46	36	55	N/A	45	11	1814
Di-n-butylphthalate	µg/kg	0	0	0	0	0	N/A	0	0	0
DIBENZO(A,H)ANTHRACENE	µg/kg	6	0	3	0	0	N/A	0	0	1229
Di-n-octylphthalate	µg/kg	0	0	0	0	0	N/A	0	0	0
FLUORANTHENE	ug/kg	129	116	93	69	87	N/A	76	14	2186
FLUORENE	µg/kg	6	0	0	0	0	N/A	0	0	22
INDENO(1,2,3-CD)PYRENE	µg/kg	16	23	24	20	19	N/A	23	5	7057
METHANAMINE, N-METHYL-N-NITROSO	µg/kg	0	0	0	0	0	N/A	0	0	0
NAPHTHALENE	µg/kg	0	0	0	0	0	N/A	0		177
PHENANTHRENE	µg/kg	91	55	38	38	39	N/A	31	7	1900
PYRENE	ug/kg	117	100	79	62	73	N/A	68	11	1414
Aroclor 1016	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1221	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1232	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1242	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1248	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1254	µg/kg	71	73	74	58	85	N/A	69	59	137
Aroclor 1260	µg/kg	128	116	120	118	126	N/A	121	38	143
Aroclor 1262	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1268	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 5432	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 5442	µg/kg	0	0	0	0	0	N/A	0	0	0

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
Aroclor 5460	ug/kg	87	89	92	58	81	N/A	70	0	112
EFH (C12-C14)	mg/kg	0	0	0	0	0	N/A	0	0	1
EFH (C15-C20)	mg/kg	0	5	2	1	7	N/A	0	0	21
EFH (C21-C30)	mg/kg	40	55	67	58	71	N/A	49	46	137
EFH (C30-C40)	mg/kg	109	109	68	54	78	N/A	42	59	73
EFH (C8-C11)	mg/kg	0	0	0	0	0	N/A	0	0	0
Oct/244 days										
Chemical	Units	Mean Concentration								
1,2,3,4,6,7,8-HEPTACHLORODIBENZO-P-DIOXIN	ng/kg	13110	9340	10086	10182	12070	11180	11160	3288	2388
1,2,3,4,6,7,8-HPCDF	ng/kg	1269	852	879	878	864	926	893	163	268
1,2,3,4,7,8,9-HPCDF	ng/kg	100	73	73	77	79	83	81	11	20
1,2,3,4,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	90	77	75	81	83	95	90	6	18
1,2,3,4,7,8-HXCDF	ng/kg	28	22	24	23	23	25	81	2	12
1,2,3,6,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	593	446	461	459	499	489	490	70	90
1,2,3,6,7,8-HXCDF	ng/kg	31	24	26	25	26	27	27	2	9
1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	214	181	178	182	197	209	197	15	38
1,2,3,7,8,9-HXCDF	ng/kg	6	0	0		0	0	0	2	0
1,2,3,7,8-PENTACHLORODIBENZOFURAN	ng/kg	10	8	9	8	7	8	7	1	4
1,2,3,7,8-PENTACHLORODIBENZO-P-DIOXIN	ng/kg	59	51	51	53	57	62	60	2	9
2,3,4,6,7,8-HXCDF	ng/kg	57	44	45	46	47	51	50	3	16
2,3,4,7,8-PECDF	ng/kg	12	9	10	10	10	11	11	1	8
2,3,7,8-TCDD	ng/kg	10	10	9	10	11	11	10	0	1
2,3,7,8-TETRACHLORODIBENZOFURAN	ng/kg	3	3	3	3	3	3	3	1	4

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
OCDD	ng/kg	108200	75060	79860	71480	82020	83840	81260	51200	26680
OCDF	ng/kg	2918	1848	1936	1898	1860	2016	1894	575	887
TCDD TEQ	ng/kg	469	269	276	282	314	314	309	62	67
1,1'-Biphenyl	µg/kg	0	0	0	0	0	0	0	0	0
1-METHYLNAPHTHALENE	µg/kg	4	2	3	2	5	3	4	0	600
2-METHYLNAPHTHALENE	µg/kg	9	10	8	9	9	9	10	0	782
ACENAPHTHENE	µg/kg	5	0	5	7	34	3	2	0	24
ACENAPHTHYLENE	µg/kg	3	1	7	0	7	2	2	0	148
ANTHRACENE	µg/kg	9	16	11	12	48	13	9	0	846
AZOBENZENE	µg/kg	0	0	0	0	0	0	0	0	0
BENZO(A)ANTHRACENE	µg/kg	21	126	38	42	96	54	29	3	1780
BENZO(A)PYRENE	µg/kg	19	87	34	40	82	49	27	0	3620
BENZO(B)FLUORANTHENE	µg/kg	47	142	66	70	127	86	58	6	5380
Benzo(e)pyrene	µg/kg	10	56	32	24	56	36	16	0	3320
BENZO(G,H,I)PERYLENE	µg/kg	12	42	19	20	51	33	17	6	2640
BENZO(K)FLUORANTHENE	µg/kg	21	57	25	28	58	32	24	0	1900
bis(2-Ethylhexyl)phthalate	µg/kg	92	79	73	51	137	56	75	20	430
Butylbenzylphthalate	µg/kg	0	0	15	0	0	15	0	0	0
Chrysene	µg/kg	59	165	83	72	144	97	73	15	2780
Di-n-butylphthalate	µg/kg	18	34	0	30	0	0	0	0	0
DIBENZO(A,H)ANTHRACENE	µg/kg	0	9	5	4	12	10	0	0	672
Di-n-octylphthalate	µg/kg	0	0	0	0	0	0	0	0	270
FLUORANTHENE	ug/kg	143	248	132	106	280	138	138	0	4540
FLUORENE	µg/kg	5	0	4	5	24	2	2	0	39
INDENO(1,2,3-CD)PYRENE	µg/kg	6	37	14	17	43	27	13	0	3500
METHANAMINE, N-METHYL-N-NITROSO	µg/kg	0	0	0	0	0	0	0	0	0
NAPHTHALENE	µg/kg	4	4	4	7	43	3	2	0	614
PHENANTHRENE	µg/kg	86	1848	75	73	229	77	74	0	4600

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
PYRENE	µg/kg	100	216	104	89	222	103	102	0	2100
Aroclor 1016	µg/kg	0	0	0	4	0	0	0	0	0
Aroclor 1221	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1232	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1242	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1248	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1254	µg/kg	65	70	63	77	77	70	78	75	29
Aroclor 1260	µg/kg	77	66	65	78	69	70	80	95	22
Aroclor 1262	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1268	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5432	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5442	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5460	µg/kg	73	61	59	81	71	77	106	91	0
Feb/362 days										
EFH (C12-C14)	mg/kg	0	0	0	0	0	0	0	0	0
EFH (C15-C20)	mg/kg	0	2	0	1	1	1	0	0	20
EFH (C21-C30)	mg/kg	39	64	47	43	42	67	55	55	109
EFH (C30-C40)	mg/kg	41	56	51	60	62	94	74	83	110
EFH (C8-C11)	mg/kg	0	0	0	0	0	0	0	0	1
Feb/0 days										
Chemical	Units	Standard Deviation								
1,2,3,4,6,7,8-HEPTACHLORODIBENZO-P-DIOXIN	ng/kg	1377	1422	1509	5018	2227	818	797	312	73
1,2,3,4,6,7,8-HPCDF	ng/kg	189	65	37	589	376	18	47	36	13
1,2,3,4,7,8,9-HPCDF	ng/kg	14	5	5	36	5	2	5	1	1
1,2,3,4,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	6	10	4	35	9	3	7	1	0
1,2,3,4,7,8-HXCDF	ng/kg	3	2	2	12	6	1	2	0	0

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
1,2,3,6,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	54	52	19	219	56	15	27	6	3
1,2,3,6,7,8-HXCDF	ng/kg	8	3	2	50	40	1	3	2	1
1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	12	24	7	83	20	2	13	2	1
1,2,3,7,8,9-HXCDF	ng/kg	1	1	1	3	3	3	3	1	2
1,2,3,7,8-PENTACHLORODIBENZOFURAN	ng/kg	1	0	1	4	2	1	1	0	0
1,2,3,7,8-PENTACHLORODIBENZO-P-DIOXIN	ng/kg	2	2	2	26	5	2	4	0	0
2,3,4,6,7,8-HXCDF	ng/kg	6	3	2	21	2	1	4	0	0
2,3,4,7,8-PECDF	ng/kg	0	1	0	5	1	1	1	0	0
2,3,7,8-TCDD	ng/kg	0	0	1	5	1	0	0	0	0
2,3,7,8-TETRACHLORODIBENZOFURAN	ng/kg	0	0	0	1	0	0	0	0	0
OCDD	ng/kg	9825	5465	5226	44883	11854	17348	6611	7244	1384
OCDF	ng/kg	428	104	119	838	133	24	92	56	53
TCDD TEQ	ng/kg	28	27	18	137	33	18	13	2	7
1,1'-Biphenyl	µg/kg	0	0	0	0	0	0	0	0	4
1-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0	0	0	0	0
2-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0	0	0	0	22
ACENAPHTHENE	µg/kg	0	0	0	0	0	0	0	36	0
ACENAPHTHYLENE	µg/kg	0	0	18	0	0	0	0	0	9
ANTHRACENE	µg/kg	10	0	13	0	0	0	14	49	48
AZOBENZENE	µg/kg	0	0	0	0	0	0	0	0	0
BENZO(A)ANTHRACENE	µg/kg	12	28	19	0	0	29	25	85	120
BENZO(A)PYRENE	µg/kg	11	24	19	17	0	29	27	98	255
BENZO(B)FLUORANTHENE	µg/kg	22	22	21	4	0	31	27	121	507
Benzo(e)pyrene	µg/kg	10	12	35	15	28	14	17	57	251
BENZO(G,H,I)PERYLENE	µg/kg	4	17	31	4	0	10	17	44	1190

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
BENZO(K)FLUORANTHENE	µg/kg	5	0	10	0	0	13	0	39	138
bis(2-Ethylhexyl)phthalate	µg/kg	0	0	0	0	0	0	0	0	0
Butylbenzylphthalate	µg/kg	0	0	0	0	0	0	0	0	0
Chrysene	µg/kg	33	15	38	0	0	35	34	112	321
Di-n-butylphthalate	µg/kg	0	0	0	0	0	0	0	0	0
DIBENZO(A,H)ANTHRACENE	µg/kg	0	0	0	0	0	0	0	14	154
Di-n-octylphthalate	µg/kg	0	0	0	0	0	0	0	0	0
FLUORANTHENE	µg/kg	60	30	99	10	4	67	88	215	156
FLUORENE	µg/kg	0	0	0	0	0	0	0	21	41
INDENO(1,2,3-CD)PYRENE	µg/kg	5	0	16	0	0	18	16	38	918
METHANAMINE, N-METHYL-N-NITROSO	µg/kg	0	0	0	0	0	0	0	0	3
NAPHTHALENE	µg/kg	0	0	0	0	0	0	0	0	19
PHENANTHRENE	µg/kg	34	23	106	22	0	61	62	224	199
PYRENE	µg/kg	55	44	60	8	33	66	73	210	97
Aroclor 1016	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1221	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1232	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1242	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1248	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1254	µg/kg	23	16	15	64	12	15	1358	5	12
Aroclor 1260	µg/kg	11	9	7	26	6	14	596	3	8
Aroclor 1262	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1268	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5432	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5442	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5460	µg/kg	52	201	86	41	11	4	2300	0	46
EFH (C12-C14)	mg/kg	0	5	0	0	2	0	2	0	2
EFH (C15-C20)	mg/kg	0	53	0	1	71	0	4	0	2

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
EFH (C21-C30)	mg/kg	43	140	5	16	252	6	22	16	15
EFH (C30-C40)	mg/kg	11	5	20	11	16	7	20	14	10
EFH (C8-C11)	mg/kg	0	3	0	0	2	0	2	0	2
Jun/126 days										
Chemical	Units	Standard Deviation								
1,2,3,4,6,7,8-HEPTACHLORODIBENZO-P-DIOXIN	ng/kg	436	1516	373	2046	783	N/A	344	237	262
1,2,3,4,6,7,8-HPCDF	ng/kg	30	46	20	66	91	N/A	34	12	18
1,2,3,4,7,8,9-HPCDF	ng/kg	3	4	2	8	9	N/A	4	2	1
1,2,3,4,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	5	6	4	7	9	N/A	3	0	1
1,2,3,4,7,8-HXCDF	ng/kg	1	3	1	1	2	N/A	1	0	1
1,2,3,6,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	11	19	18	31	37	N/A	10	2	4
1,2,3,6,7,8-HXCDF	ng/kg	1	3	2	1	4	N/A	2	0	1
1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	6	15	7	15	14	N/A	5	1	2
1,2,3,7,8,9-HXCDF	ng/kg	3	1	4	4	4	N/A	3	0	1
1,2,3,7,8-PENTACHLORODIBENZOFURAN	ng/kg	1	1	1	1	1	N/A	1	0	1
1,2,3,7,8-PENTACHLORODIBENZO-P-DIOXIN	ng/kg	3	1	4	2	3	N/A	3	0	4
2,3,4,6,7,8-HXCDF	ng/kg	1	2	2	2	4	N/A	2	0	1
2,3,4,7,8-PECDF	ng/kg	2	1	1	1	1	N/A	1	0	1
2,3,7,8-TCDD	ng/kg	2	2	1	0	1	N/A	1	0	0
2,3,7,8-TETRACHLORODIBENZOFURAN	ng/kg	0	0	0	0	1	N/A	0	0	0
OCDD	ng/kg	3454	13006	21351	18368	12520	N/A	1632	6003	2859
OCDF	ng/kg	40	50	26618	149	203	N/A	132	34	64
TCDD TEQ	ng/kg	20	27	635	34	19	N/A	5	5	5

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
1,1'-Biphenyl	µg/kg	0	0	0	0	0	N/A	0	0	4
1-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0	N/A	0	0	5
2-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0	N/A	0	0	8
ACENAPHTHENE	µg/kg	21	0	0	0	0	N/A	0	0	6
ACENAPHTHYLENE	µg/kg	0	7	0	0	0	N/A	0	0	11
ANTHRACENE	ug/kg	31	9	7	10	0	N/A	0	0	48
AZOBENZENE	µg/kg	0	0	0	0	0	N/A	0	0	0
BENZO(A)ANTHRACENE	µg/kg	64	25	27	11	29	N/A	15	12	111
BENZO(A)PYRENE	µg/kg	56	10	24	8	23	N/A	13	14	270
BENZO(B)FLUORANTHENE	µg/kg	70	22	29	7	40	N/A	16	22	486
Benzo(e)pyrene	µg/kg	30	7	17	7	19	N/A	11	16	248
BENZO(G,H,I)PERYLENE	µg/kg	29	5	16	4	11	N/A	9	10	577
BENZO(K)FLUORANTHENE	µg/kg	23	8	12	7	18	N/A	10	6	92
bis(2-Ethylhexyl)phthalate	µg/kg	0	0	0	0	0	N/A	0	0	0
Butylbenzylphthalate	µg/kg	0	0	0	0	0	N/A	0	0	0
Chrysene	µg/kg	69	23	25	10	26	N/A	16	25	135
Di-n-butylphthalate	µg/kg	0	0	0	0	0	N/A	0	0	0
DIBENZO(A,H)ANTHRACENE	µg/kg	10	0	7	0	0	N/A	0	0	138
Di-n-octylphthalate	µg/kg	0	0	0	0	0	N/A	0	0	0
FLUORANTHENE	µg/kg	158	42	49	22	48	N/A	26	32	168
FLUORENE	µg/kg	13	0	0	0	0	N/A	0	0	3
INDENO(1,2,3-CD)PYRENE	µg/kg	29	6	12	3	15	N/A	8	11	326
METHANAMINE, N-METHYL-N-NITROSO	µg/kg	0	0	0	0	0	N/A	0	0	0
NAPHTHALENE	µg/kg	0	0	0	0	0	N/A	0		6
PHENANTHRENE	µg/kg	139	20	19	23	25	N/A	9	15	153
PYRENE	µg/kg	142	34	45	20	39	N/A	23	25	135
Aroclor 1016	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1221	µg/kg	0	0	0	0	0	N/A	0	0	0

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
Aroclor 1232	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1242	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1248	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1254	µg/kg	19	6	28	8	12	N/A	12	4	23
Aroclor 1260	µg/kg	8	13	7	4	11	N/A	7	2	13
Aroclor 1262	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 1268	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 5432	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 5442	µg/kg	0	0	0	0	0	N/A	0	0	0
Aroclor 5460	ug/kg	59	47	72	3	29	N/A	20	0	17
EFH (C12-C14)	mg/kg	0	0	0	0	0	N/A	0	0	2
EFH (C15-C20)	mg/kg	0	7	3	1	4	N/A	0	0	2
EFH (C21-C30)	mg/kg	12	10	22	14	7	N/A	5	10	14
EFH (C30-C40)	mg/kg	42	38	21	12	25	N/A	8	18	15
EFH (C8-C11)	mg/kg	0	0	0	0	0	N/A	0	0	1
Oct/244 days										
Chemical	Units	Standard Deviation								
1,2,3,4,6,7,8-HEPTACHLORODIBENZO-P-DIOXIN	ng/kg	6077	758	1718	665	2901	554	1078	569	229
1,2,3,4,6,7,8-HPCDF	ng/kg	827	22	113	78	19	29	48	27	15
1,2,3,4,7,8,9-HPCDF	ng/kg	54	1	8	4	2	4	4	1	1
1,2,3,4,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	22	1	6	3	2	2	8	1	1
1,2,3,4,7,8-HXCDF	ng/kg	11	1	3	2	1	2	4	0	3
1,2,3,6,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	257	23	51	24	59	10	38	9	5
1,2,3,6,7,8-HXCDF	ng/kg	12	1	2	1	1	1	1	0	1
1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	54	4	13	5	19	4	12	2	2

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
1,2,3,7,8,9-HXCDF	ng/kg	5	0	0	0	0	0	0	2	0
1,2,3,7,8-PENTACHLORODIBENZOFURAN	ng/kg	3	1	1	1	1	1	1	0	1
1,2,3,7,8-PENTACHLORODIBENZO-P-DIOXIN	ng/kg	11	1	3	2	2	1	6	0	1
2,3,4,6,7,8-HXCDF	ng/kg	27	2	4	3	0	2	3	0	1
2,3,4,7,8-PECDF	ng/kg	5	2	2	0	0	1	1	0	2
2,3,7,8-TCDD	ng/kg	2	1	1	1	1	1	2	0	0
2,3,7,8-TETRACHLORODIBENZOFURAN	ng/kg	1	0	0	0	0	0	1	0	0
OCDD		48267	4678	9008	13162	14696	6723	18256	11601	2100
OCDF	ng/kg	2154	66	338	240	64	105	73	97	65
TCDD TEQ	ng/kg	270	10	28	11	41	8	30	11	4
1,1'-Biphenyl	µg/kg	0	0	0	0	0	0	0	0	0
1-METHYLNAPHTHALENE	µg/kg	8	4	6	4	10	4	5	0	60
2-METHYLNAPHTHALENE	µg/kg	9	3	9	5	10	1	6	0	77
ACENAPHTHENE	µg/kg	11	0	7	15	76	8	5	0	3
ACENAPHTHYLENE	µg/kg	8	2	7	0	3	3	5	0	16
ANTHRACENE	µg/kg	11	18	9	9	85	10	4	0	88
AZOBENZENE	µg/kg	0	0	0	0	0	0	0	0	0
BENZO(A)ANTHRACENE	µg/kg	13	209	24	27	148	34	11	8	259
BENZO(A)PYRENE	µg/kg	7	131	19	25	128	30	9	0	614
BENZO(B)FLUORANTHENE	µg/kg	29	195	34	34	154	40	12	5	858
Benzo(e)pyrene	µg/kg	21	83	33	34	74	38	22	0	432
BENZO(G,H,I)PERYLENE	µg/kg	3	50	7	10	67	18	3	6	288
BENZO(K)FLUORANTHENE	µg/kg	16	81	13	15	75	16	6	0	255
bis(2-Ethylhexyl)phthalate	µg/kg	87	48	42	47	261	53	44	45	207
Butylbenzylphthalate	µg/kg	0	0	34	0	0	33	0	0	0
Chrysene	µg/kg	51	232	57	33	148	40	20	3	460
Di-n-butylphthalate	µg/kg	41	76	0	67	0	0	0	0	0

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
DIBENZO(A,H)ANTHRACENE	µg/kg	0	21	5	6	22	8	0	0	81
Di-n-octylphthalate	µg/kg	0	0	0	0	0	0	0	0	48
FLUORANTHENE	µg/kg	217	371	90	60	325	64	85	0	662
FLUORENE	µg/kg	11	0	5	12	54	5	5	0	5
INDENO(1,2,3-CD)PYRENE	µg/kg	5	52	6	11	65	16	3	0	367
METHANAMINE, N-METHYL-N-NITROSO	µg/kg	0	0	0	0	0	0	0	0	0
NAPHTHALENE	µg/kg	6	5	5	4	65	4	4	0	50
PHENANTHRENE	µg/kg	142	66	71	68	359	40	53	0	534
PYRENE	µg/kg	145	339	72	55	273	45	59	0	255
Aroclor 1016	µg/kg	0	0	0	9	0	0	0	0	0
Aroclor 1221	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1232	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1242	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1248	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1254	µg/kg	15	15	22	13	35	7	11	31	2
Aroclor 1260	µg/kg	8	16	12	4	6	5	9	11	2
Aroclor 1262	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1268	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5432	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5442	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5460	µg/kg	23	7	10	12	9	25	30	53	0
Feb/362 days										
EFH (C12-C14)	mg/kg	0	0	0	0	0	0	0	0	0
EFH (C15-C20)	mg/kg	0	4	0	3	2	2	0	0	3
EFH (C21-C30)	mg/kg	9	26	5	5	9	69	18	23	20
EFH (C30-C40)	mg/kg	13	25	7	6	12	93	22	19	32
EFH (C8-C11)	mg/kg	0	0	0	0	0	0	0	0	1
Feb/0 days										

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
Chemical	Units	Standard Error of the Mean								
1,2,3,4,6,7,8-HEPTACHLORODIBENZO-P-DIOXIN	ng/kg	616	636	675	2244	996	366	356	140	32
1,2,3,4,6,7,8-HPCDF	ng/kg	85	29	17	263	168	8	21	16	6
1,2,3,4,7,8,9-HPCDF	ng/kg	6	2	2	16	2	1	2	0	0
1,2,3,4,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	3	5	2	16	4	1	3	0	0
1,2,3,4,7,8-HXCDF	ng/kg	1	1	1	5	3	0	1	0	0
1,2,3,6,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	24	23	9	98	25	7	12	3	1
1,2,3,6,7,8-HXCDF	ng/kg	3	1	1	22	18	1	1	1	1
1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	5	11	3	37	9	1	6	1	0
1,2,3,7,8,9-HXCDF	ng/kg	0	0	0	1	1	1	1	0	1
1,2,3,7,8-PENTACHLORODIBENZOFURAN	ng/kg	0	0	1	2	1	0	0	0	0
1,2,3,7,8-PENTACHLORODIBENZO-P-DIOXIN	ng/kg	1	1	1	11	2	1	2	0	0
2,3,4,6,7,8-HXCDF	ng/kg	3	1	1	9	1	0	2	0	0
2,3,4,7,8-PECDF	ng/kg	0	0	0	2	0	0	1	0	0
2,3,7,8-TCDD	ng/kg	0	0	0	2	0	0	0	0	0
2,3,7,8-TETRACHLORODIBENZOFURAN	ng/kg	0	0	0	1	0	0	0	0	0
OCDD		4394	2444	2337	20072	5301	7758	2956	3240	619
OCDF	ng/kg	191	46	53	375	59	11	41	25	24
TCDD TEQ	ng/kg	13	12	8	61	15	8	6	3	1
1,1'-Biphenyl	µg/kg	0	0	0	0	0	0	0	0	2
1-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0	0	0	0	0
2-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0	0	0	0	10
ACENAPHTHENE	µg/kg	0	0	0	0	0	0	0	16	0

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
ACENAPHTHYLENE	µg/kg	0	0	8	0	0	0	0	0	4
ANTHRACENE	ug/kg	4	0	6	0	0	0	6	22	21
AZOBENZENE	µg/kg	0	0	0	0	0	0	0	0	0
BENZO(A)ANTHRACENE	µg/kg	5	13	9	0	0	13	11	38	54
BENZO(A)PYRENE	µg/kg	5	11	8	7	0	13	12	44	114
BENZO(B)FLUORANTHENE	µg/kg	10	10	9	2	0	14	12	54	227
Benzo(e)pyrene	µg/kg	4	5	16	7	12	6	8	26	112
BENZO(G,H,I)PERYLENE	µg/kg	2	8	14	2	0	5	8	20	532
BENZO(K)FLUORANTHENE	µg/kg	2	0	4	0	0	6	0	18	62
bis(2-Ethylhexyl)phthalate	µg/kg	0	0	0	0	0	0	0	0	0
Butylbenzylphthalate	µg/kg	0	0	0	0	0	0	0	0	0
Chrysene	µg/kg	15	7	17	0	0	16	15	50	143
Di-n-butylphthalate	µg/kg	0	0	0	0	0	0	0	0	0
DIBENZO(A,H)ANTHRACENE	µg/kg	0	0	0	0	0	0	0	6	69
Di-n-octylphthalate	µg/kg	0	0	0	0	0	0	0	0	0
FLUORANTHENE	µg/kg	27	14	44	5	2	30	39	96	70
FLUORENE	µg/kg	0	0	0	0	0	0	0	10	18
INDENO(1,2,3-CD)PYRENE	µg/kg	2	0	7	0	0	8	7	17	411
METHANAMINE, N-METHYL-N-NITROSO	µg/kg	0	0	0	0	0	0	0	0	1
NAPHTHALENE	µg/kg	0	0	0	0	0	0	0	0	9
PHENANTHRENE	ug/kg	15	10	47	10	0	27	28	100	89
PYRENE	ug/kg	24	20	27	3	15	29	33	94	43
Aroclor 1016	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1221	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1232	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1242	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1248	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1254	µg/kg	10	7	7	29	5	7	607	2	5

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
Aroclor 1260	µg/kg	5	4	3	12	3	6	266	1	3
Aroclor 1262	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1268	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5432	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5442	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5460	ug/kg	23	90	38	18	5	2	1029	0	21
EFH (C12-C14)	mg/kg	0	2	0	0	1	0	1	0	1
EFH (C15-C20)	mg/kg	0	24	0	1	32	0	2	0	1
EFH (C21-C30)	mg/kg	19	63	2	7	113	3	10	7	7
EFH (C30-C40)	mg/kg	5	2	9	5	7	3	9	6	5
EFH (C8-C11)	mg/kg	0	1	0	0	1	0	1	0	1
Jun/126 days										
Chemical	Units	Standard Error of the Mean								
1,2,3,4,6,7,8-HEPTACHLORODIBENZO-P-DIOXIN	ng/kg	195	678	167	915	350		130	106	99
1,2,3,4,6,7,8-HPCDF	ng/kg	14	21	9	29	41		13	5	7
1,2,3,4,7,8,9-HPCDF	ng/kg	1	2	1	4	4		1	1	0
1,2,3,4,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	2	3	2	3	4		1	0	0
1,2,3,4,7,8-HXCDF	ng/kg	1	1	1	0	1		0	0	0
1,2,3,6,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	5	9	8	14	17		4	1	1
1,2,3,6,7,8-HXCDF	ng/kg	0	1	1	0	2		1	0	0
1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	2	7	3	7	6		2	0	1
1,2,3,7,8,9-HXCDF	ng/kg	1	1	2	2	2		1	0	0
1,2,3,7,8-PENTACHLORODIBENZOFURAN	ng/kg	0	0	0	0	0		0	0	0

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
1,2,3,7,8-PENTACHLORODIBENZO-P-DIOXIN	ng/kg	1	1	2	1	1		1	0	1
2,3,4,6,7,8-HXCDF	ng/kg	0	1	1	1	2		1	0	0
2,3,4,7,8-PECDF	ng/kg	1	1	0	0	0		1	0	0
2,3,7,8-TCDD	ng/kg	1	1	0	0	1		0	0	0
2,3,7,8-TETRACHLORODIBENZOFURAN	ng/kg	0	0	0	0	1		0	0	0
OCDD	ng/kg	1545	5817	9548	8214	5599		617	2685	1081
OCDF	ng/kg	18	22	11904	67	91		50	15	24
TCDD TEQ	ng/kg	9	12	284	15	8		2	2	2
1,1'-Biphenyl	µg/kg	0	0	0	0	0		0	0	1
1-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0		0	0	2
2-METHYLNAPHTHALENE	µg/kg	0	0	0	0	0		0	0	3
ACENAPHTHENE	µg/kg	9	0	0	0	0		0	0	2
ACENAPHTHYLENE	µg/kg	0	3	0	0	0		0	0	4
ANTHRACENE	µg/kg	14	4	3	5	0		0	0	18
AZOBENZENE	µg/kg	0	0	0	0	0		0	0	0
BENZO(A)ANTHRACENE	µg/kg	29	11	12	5	13		5	5	42
BENZO(A)PYRENE	µg/kg	25	4	11	3	10		5	6	102
BENZO(B)FLUORANTHENE	µg/kg	31	10	13	3	18		6	10	184
Benzo(e)pyrene	µg/kg	13	3	8	3	8		4	7	94
BENZO(G,H,I)PERYLENE	µg/kg	13	2	7	2	5		4	5	218
BENZO(K)FLUORANTHENE	µg/kg	10	4	5	3	8		4	3	35
bis(2-Ethylhexyl)phthalate	µg/kg	0	0	0	0	0		0	0	0
Butylbenzylphthalate	µg/kg	0	0	0	0	0		0	0	0
Chrysene	µg/kg	31	11	11	4	12		6	11	51
Di-n-butylphthalate	µg/kg	0	0	0	0	0		0	0	0
DIBENZO(A,H)ANTHRACENE	µg/kg	4	0	3	0	0		0	0	52
Di-n-octylphthalate	µg/kg	0	0	0	0	0		0	0	0

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
FLUORANTHENE	ug/kg	71	19	22	10	21		10	14	63
FLUORENE	µg/kg	6	0	0	0	0		0	0	1
INDENO(1,2,3-CD)PYRENE	µg/kg	13	3	6	2	7		3	5	123
METHANAMINE, N-METHYL-N-NITROSO	µg/kg	0	0	0	0	0		0	0	0
NAPHTHALENE	µg/kg	0	0	0	0	0		0		3
PHENANTHRENE	µg/kg	62	9	9	10	11		3	7	58
PYRENE	µg/kg	64	15	20	9	17		9	11	51
Aroclor 1016	µg/kg	0	0	0	0	0		0	0	0
Aroclor 1221	µg/kg	0	0	0	0	0		0	0	0
Aroclor 1232	µg/kg	0	0	0	0	0		0	0	0
Aroclor 1242	µg/kg	0	0	0	0	0		0	0	0
Aroclor 1248	µg/kg	0	0	0	0	0		0	0	0
Aroclor 1254	µg/kg	8	3	13	4	5		5	2	9
Aroclor 1260	µg/kg	4	6	3	2	5		607	1	5
Aroclor 1262	µg/kg	0	0	0	0	0		0	0	0
Aroclor 1268	µg/kg	0	0	0	0	0		0	0	0
Aroclor 5432	µg/kg	0	0	0	0	0		0	0	0
Aroclor 5442	µg/kg	0	0	0	0	0		0	0	0
Aroclor 5460	µg/kg	26	21	32	1	13		7	0	7
EFH (C12-C14)	mg/kg	0	0	0	0	0		0	0	1
EFH (C15-C20)	mg/kg	0	3	1	1	2		0	0	1
EFH (C21-C30)	mg/kg	5	4	10	6	3		2	4	5
EFH (C30-C40)	mg/kg	19	17	9	6	11		3	8	6
EFH (C8-C11)	mg/kg	0	0	0	0	0		0	0	0
Oct/244 days										
Chemical	Units	Standard Error of the Mean								

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
1,2,3,4,6,7,8-HEPTACHLORODIBENZO-P-DIOXIN	ng/kg	2718	339	768	297	1297	248	482	255	103
1,2,3,4,6,7,8-HPCDF	ng/kg	370	10	51	35	8	13	22	12	7
1,2,3,4,7,8,9-HPCDF	ng/kg	24	1	3	2	1	2	2	1	0
1,2,3,4,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	10	1	3	2	1	1	3	0	0
1,2,3,4,7,8-HXCDF	ng/kg	5	0	1	1	0	1	2	1	1
1,2,3,6,7,8-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	115	10	23	11	26	5	17	4	2
1,2,3,6,7,8-HXCDF	ng/kg	5	0	1	1	1	0	0	0	0
1,2,3,7,8,9-HEXACHLORODIBENZO-P-DIOXIN	ng/kg	24	2	6	2	8	2	6	1	1
1,2,3,7,8,9-HXCDF	ng/kg	2	0	0	0	0	0	0	1	0
1,2,3,7,8-PENTACHLORODIBENZOFURAN	ng/kg	1	0	0	1	0	0	0	0	0
1,2,3,7,8-PENTACHLORODIBENZO-P-DIOXIN	ng/kg	5	1	1	1	1	1	3	0	0
2,3,4,6,7,8-HXCDF	ng/kg	12	1	2	1	0	1	2	0	0
2,3,4,7,8-PECDF	ng/kg	2	1	1	0	0	0	0	0	1
2,3,7,8-TCDD	ng/kg	1	0	0	1	0	0	1	0	0
2,3,7,8-TETRACHLORODIBENZOFURAN	ng/kg	0	0	0	0	0	0	0	0	0
OCDD	ng/kg	21586	2092	4029	5886	6572	3006	8164	5188	939
OCDF	ng/kg	963	30	151	107	29	47	33	43	29
TCDD TEQ	ng/kg	121	4	12	5	18	4	13	5	2
1,1'-Biphenyl	µg/kg	0	0	0	0	0	0	0	0	0
1-METHYLNAPHTHALENE	µg/kg	4	2	3	2	5	2	2	0	27
2-METHYLNAPHTHALENE	µg/kg	4	1	4	2	4	1	3	0	35
ACENAPHTHENE	µg/kg	5	0	3	7	34	3	2	0	1
ACENAPHTHYLENE	µg/kg	3	1	3	0	1	2	2	0	7

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
ANTHRACENE	µg/kg	5	8	4	4	38	4	2	0	40
AZOBENZENE	µg/kg	0	0	0	0	0	0	0	0	0
BENZO(A)ANTHRACENE	µg/kg	6	94	11	12	66	15	5	3	116
BENZO(A)PYRENE	µg/kg	3	58	8	11	57	13	4	0	275
BENZO(B)FLUORANTHENE	µg/kg	13	87	15	15	69	18	5	2	384
Benzo(e)pyrene	µg/kg	10	37	15	15	33	17	10	0	193
BENZO(G,H,I)PERYLENE	µg/kg	1	22	3	5	30	8	1	3	129
BENZO(K)FLUORANTHENE	µg/kg	7	36	6	7	33	7	3	0	114
bis(2-Ethylhexyl)phthalate	µg/kg	39	22	19	21	117	24	20	20	93
Butylbenzylphthalate	µg/kg	0	0	15	0	0	15	0	0	0
Chrysene	µg/kg	23	104	25	15	66	18	9	1	206
Di-n-butylphthalate	µg/kg	18	34	0	30	0	0	0	0	0
DIBENZO(A,H)ANTHRACENE	µg/kg	0	9	2	3	10	4	0	0	36
Di-n-octylphthalate	µg/kg	0	0	0	0	0	0	0	0	22
FLUORANTHENE	µg/kg	97	166	40	27	145	29	38	0	296
FLUORENE	µg/kg	5	0	2	5	24	2	2	0	2
INDENO(1,2,3-CD)PYRENE	ug/kg	2	23	3	5	29	7	1	0	164
METHANAMINE, N-METHYL-N-NITROSO	µg/kg	0	0	0	0	0	0	0	0	0
NAPHTHALENE	µg/kg	3	2	2	2	29	2	2	0	22
PHENANTHRENE	µg/kg	64	30	32	30	161	18	24	0	239
PYRENE	µg/kg	65	152	32	24	122	20	26	0	114
Aroclor 1016	µg/kg	0	0	0	4	0	0	0	0	0
Aroclor 1221	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1232	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1242	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1248	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1254	µg/kg	7	7	10	6	16	3	5	14	1
Aroclor 1260	µg/kg	4	7	5	2	3	2	4	5	1

Treatment		NUTR	SOLE	RICE	AUGM	COMB	STER	UNAA	UNAC	UNAB
Aroclor 1262	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 1268	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5432	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5442	µg/kg	0	0	0	0	0	0	0	0	0
Aroclor 5460	µg/kg	10	3	4	5	4	11	14	24	0
Feb/362 days										
EFH (C12-C14)	mg/kg	0	0	0	0	0	0	0	0	0
EFH (C15-C20)	mg/kg	0	2	0	1	1	1	0	0	1
EFH (C21-C30)	mg/kg	4	11	2	2	4	31	8	10	9
EFH (C30-C40)	mg/kg	6	11	3	3	5	42	10	8	14
EFH (C8-C11)	mg/kg	0	0	0	0	0	0	0	0	1

Table I-2: Total EFH concentration in microcosms during incubation

	Time	NUTR	SOLE	RICE	AUGM	COMB	UNAA	UNAB	UNAC	STER
Average (mg/kg)	Feb/0 days	182	1640	154	146	1980	152	230	100	101
	Jun/126 days	152	172	137	113	156	89	226	105	N/A
	Feb/362 days	81	123	99	106	105	131	240	139	162
Standard Deviation (mg/kg)	Feb/0 days	51	219	21	23	327	55	12	29	9
	Jun/126 days	54	54	46	27	32	12	30	26	N/A
	Feb/362 days	23	55	12	12	21	39	53	38	161
Standard Error (mg/kg)	Feb/0 days	23	98	9	10	146	25	5	13	4
	Jun/126 days	24	24	20	12	14	5	14	12	N/A
	Feb/362 days	10	25	5	5	10	17	24	17	72

Table I-3: Total PAH concentration in microcosms during incubation

	Time	NUTR	SOLE	RICE	AUGM	COMB	UNAA	UNAB	UNAC	STER
Average (µg/kg)	Feb/0 days	626	350	714	214	87	467	45139	626	523
	Jun/126 days	727	538	489	382	485	429	39238	153	N/A
	Oct/244 days	673	1390	759	710	1672	684	40585	50	943
Standard Deviation (µg/kg)	Feb/0 days	228	191	414	34	56	297	3441	1361	364
	Jun/126 days	882	195	282	113	282	158	1746	211	N/A
	Oct/244 days	714	1812	372	479	2014	224	5198	50	289
Standard Error (µg/kg)	Feb/0 days	102	85	185	15	25	133	1539	609	163
	Jun/126 days	394	87	126	50	126	71	781	94	N/A
	Oct/244 days	319	810	166	214	901	100	2325	23	129

Table I-4: Aroclor 1260, 5460, and 1254 concentrations in microcosms during incubation

AROCLOR 1260		NUTR	SOLE	RICE	AUGM	COMB	UNAA	UNAB	UNAC	STER
	Feb/0 days	97	108	110	110	103	328	111	37	112
	Jun/126 days	128	116	120	118	126	121	143	38	N/A
Average (µg/kg)	Oct/244 days	77	66	65	78	69	80	22	95	70
	Feb/0 days	11	9	7	26	6	596	8	3	14
	Jun/126 days	8	13	7	4	11	7	13	2	N/A
Standard Deviation (µg/kg)	Oct/244 days	8	16	12	4	6	9	2	11	5
	Feb/0 days	5	4	3	12	3	266	3	1	6
	Jun/126 days	4	6	3	2	5	607	5	1	N/A
Standard Error (µg/kg)	Oct/244 days	4	7	5	2	3	4	1	5	2
AROCLOR 5460		NUTR	SOLE	RICE	AUGM	COMB	UNAA	UNAB	UNAC	STER
	Feb/0 days	97	191	142	108	93	908	102	0	80
	Jun/126 days	87	89	92	58	81	70	112	0	N/A
Average (µg/kg)	Oct/244 days	73	61	59	81	71	106	0	91	77
	Feb/0 days	52	201	86	41	11	2300	46	0	4
	Jun/126 days	59	47	72	3	29	20	17	0	
Standard Deviation (µg/kg)	Oct/244 days	23	7	10	12	9	30	0	53	25
	Feb/0 days	23	90	38	18	5	1029	21	0	2
	Jun/126 days	26	21	32	1	13	7	7	0	
Standard Error (µg/kg)	Oct/244 days	10	3	4	5	4	14	0	24	11

AROCLOR 1254		NUTR	SOLE	RICE	AUGM	COMB	UNAA	UNAB	UNAC	STER
	Feb/0 days	132	150	142	160	140	645	127	59	132
	Jun/126 days	71	73	74	58	85	69	137	59	
Average (µg/kg)	Oct/244 days	65	70	63	77	77	78	29	75	70
	Feb/0 days	23	16	15	64	12	1358	12	5	15
	Jun/126 days	19	6	28	8	12	12	23	4	
Standard Deviation (µg/kg)	Oct/244 days	15	15	22	13	35	11	2	31	7
	Feb/0 days	10	7	7	29	5	607	5	2	7
	Jun/126 days	8	3	13	4	5	5	9	2	
Standard Error (µg/kg)	Oct/244 days	7	7	10	6	16	5	1	14	3

Table I-5: Total dioxin concentration in microcosms during incubation

	Time	NUTR	SOLE	RICE	AUGM	COMB	UNAA	UNAB	UNAC	STER
Average (ng/kg)	Feb/0 days	98898	99547	89064	116316	100358	99432	26581	54509	91803
	Jun/126 days	79230	84227	85548	90113	88368	81967	26041	54526	N/A
	Oct/244 days	126710	88048	93723	85415	97854	96257	30452	55342	99035
Standard Deviation (ng/kg)	Feb/0 days	11141	6749	6327	51418	13966	9032	1536	7608	18189
	Jun/126 days	3779	14579	19916	20613	12666	2047	2396	6219	N/A
	Oct/244 days	57719	4316	11257	85415	17633	19335	2397	12275	7052
Standard Error (ng/kg)	Feb/0 days	4982	3018	2830	22995	6246	4039	687	3403	8135
	Jun/126 days	1690	6520	8907	9219	5665	915	1072	2781	N/A
	Oct/244 days	25813	1930	5034	38199	7886	8647	1072	5490	3154

Table I-6: TCDD TEQ in microcosms during incubation

	Time	NUTR	SOLE	RICE	AUGM	COMB	UNAA	UNAB	UNAC	STER
Average (ng/kg)	Feb/0 days	297	303	267	332	286	288	57	55	266
	Jun/126 days	247	264	250	266	262	264	54	56	N/A
	Oct/244 days	247	264	276	282	314	309	67	62	314
Standard Deviation (ng/kg)	Feb/0 days	28	27	18	137	33	13	2	7	18
	Jun/126 days	20	27	15	34	19	4	2	5	N/A
	Oct/244 days	137	10	28	11	41	30	4	11	8
Standard Error (ng/kg)	Feb/0 days	13	12	8	61	15	6	1	3	8
	Jun/126 days	9	12	7	15	8	2	1	2	N/A
	Oct/244 days	61	4	12	5	18	13	2	5	4